

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
5 December 2002 (05.12.2002)

PCT

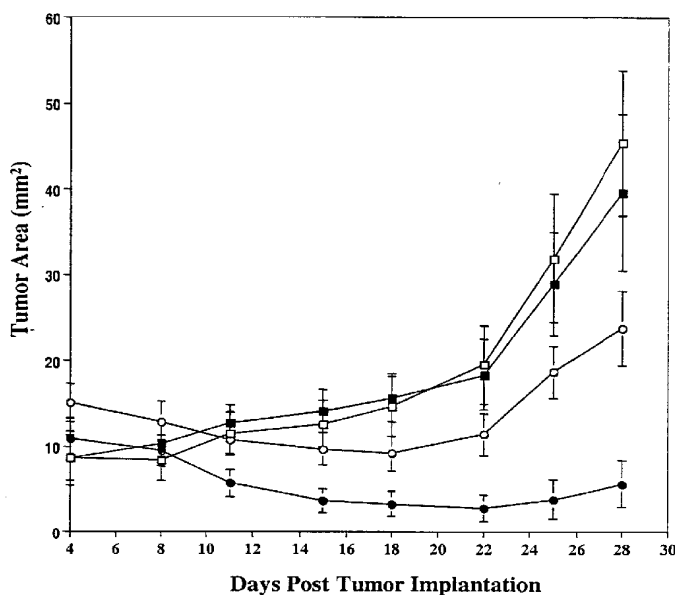
(10) International Publication Number  
**WO 02/097033 A2**

- (51) International Patent Classification<sup>7</sup>: **C12N** 60/369,860 5 April 2002 (05.04.2002) US
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- (22) International Filing Date: 7 May 2002 (07.05.2002)
- (25) Filing Language: English (72) Inventors; and
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- (30) Priority Data:
- |            |                                |    |
|------------|--------------------------------|----|
| 60/293,473 | 25 May 2001 (25.05.2001)       | US |
| 60/294,981 | 4 June 2001 (04.06.2001)       | US |
| 60/309,176 | 2 August 2001 (02.08.2001)     | US |
| 60/323,807 | 21 September 2001 (21.09.2001) | US |
| 60/327,364 | 9 October 2001 (09.10.2001)    | US |
| 60/331,044 | 7 November 2001 (07.11.2001)   | US |
| 60/331,310 | 14 November 2001 (14.11.2001)  | US |
| 60/341,237 | 20 December 2001 (20.12.2001)  | US |

[Continued on next page]

(54) Title: ANTIBODIES THAT IMMUNOSPECIFICALLY BIND TO TRAIL RECEPTORS

Effect of T1014A04 on SW480 tumor growth in Swiss Nu/Nu mice



n=6

—□— PBS

—■— T1014A04

—○— Topotecan (0.3mg/kg)

—●— Topo (0.3mg/kg) + 14A04

(57) Abstract: The present invention relates to antibodies and related molecules that immunospecifically bind to TRAIL receptor, TR4. Such antibodies have uses, for example, in the prevention and treatment of cancers and other proliferative disorders. The invention also relates to nucleic acid molecules encoding anti-TR4 antibodies, vectors, and host cells containing these nucleic acids, and methods for producing the same. The present invention relates to methods and compositions for preventing, detecting, diagnosing, treating, or ameliorating a disease or disorder, especially cancer and other hyperproliferative disorders, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TRAIL receptor TR4.



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(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Antibodies that Immunospecifically Bind to TRAIL Receptors**

**Field of the Invention**

[0001] The present invention relates to antibodies and related molecules that immunospecifically bind to TRAIL receptor, TR4. Such antibodies have uses, for example, in the prevention and treatment of cancers and other proliferative disorders. The invention also relates to nucleic acid molecules encoding anti-TR4 antibodies, vectors and host cells containing these nucleic acids, and methods for producing the same. The present invention relates to methods and compositions for preventing, detecting, diagnosing, treating or ameliorating a disease or disorder, especially cancer and other hyperproliferative disorders, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TR4.

**Background of the Invention**

[0002] Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

[0003] For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, at least eighteen members of the TNF ligand superfamily have been identified and at least nineteen members of the TNF-receptor superfamily have been characterized (*See, e.g., Locksley et al., Cell* (2001) 104:487-501).

[0004] Among the ligands there are included TNF- $\alpha$ , lymphotoxin- $\alpha$  (LT- $\alpha$ , also known as TNF- $\beta$ ), LT- $\beta$  (found in complex heterotrimer LT- $\alpha$ 2- $\beta$ ), FasL, CD40L, CD27L, CD30L, 4-IBBL, OX40L and nerve growth factor (NGF). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-IBB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals*, 22:291-295 (1994)).

[0005] Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

[0006] Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., *et al.*, *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. *et al.*, *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (Lee, K.F. *et al.*, *Cell* 69:737 (1992)).

[0007] TNF and LT- $\alpha$  are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT- $\alpha$ , acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT- $\alpha$  are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science* 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

[0008] Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia *et al.*, *Cell* 74:845 (1993)).

[0009] Apoptosis, or programmed cell death, is a physiologic process essential to the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267, 1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267, 1456-1462 (1995)). Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J.L. Cleveland, *et al.*, *Cell* 81, 479-482 (1995);



A. Fraser, *et al.*, *Cell* 85, 781-784 (1996); S. Nagata, *et al.*, *Science* 267, 1449-56 (1995)). Both are members of the TNF receptor family which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C.A. Smith, *et al.*, *Science* 248, 1019-23 (1990); M. Tewari, *et al.*, in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman and Hall, London, 1995). While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the "death domain", which is distantly related to the *Drosophila* suicide gene, reaper (P. Golstein, *et al.*, *Cell* 81, 185-6 (1995); K. White *et al.*, *Science* 264, 677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A.M. Chinnaiyan, *et al.*, *Cell* 81, 505-12 (1995); M. P. Boldin, *et al.*, *J. Biol Chem* 270, 7795-8 (1995); F.C. Kischkel, *et al.*, *EMBO* 14, 5579-5588 (1995)), which in turn binds and presumably activates FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio *et al.*, *Cell* 85, 817-827 (1996); M.P. Boldin, *et al.*, *Cell* 85, 803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF-kB (L.A. Tartaglia, *et al.*, *Immunol Today* 13, 151-3 (1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD, also contains a death domain (H. Hsu, *et al.*, *Cell* 81, 495-504 (1995); H. Hsu, *et al.*, *Cell* 84, 299-308 (1996)). Through its associations with a number of signaling molecules including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF-kB activation (H. Hsu, *et al.*, *Cell* 84, 299-308 (1996); H. Hsu, *et al.*, *Immunity* 4, 387-396 (1996)).

**[0010]** One TNF-related apoptosis inducing ligand has been reported by several groups and has been ascribed the name Apoptosis Inducing Molecule I (AIM-I) (Intention Application No. WO 97/33899) and TNF-related apoptosis-inducing ligand or (TRAIL) (Wiley, S.R. et al., *Immunity* 3:673-682 (1995)). Pitti, R.M. et al., refer to the new molecule as Apo-2 ligand or ("Apo-2L"). For convenience, it will be referred to herein as TRAIL. The amino acid sequence of TRAIL is given in SEQ ID NO:66.

**[0011]** Unlike FAS ligand whose transcripts appear to be largely restricted to stimulated T-cells, significant levels of TRAIL are seen in many tissues, and it is constitutively transcribed by some cell lines. It has been shown that TRAIL acts

independently from FAS ligand (Wiley, S.R., et al. (1995)), *supra*). Studies by Marsters, S.A. et al., have indicated that TRAIL activates apoptosis rapidly, within a time frame that is similar to death signalling by FAS/Apo-1L but much faster than TNF-induced apoptosis (*Current Biology*, 6:750-752 (1996)).

**[0012]** As many as five TRAIL receptors have been identified, including TR4 (also known as TRAIL receptor 1 (TRAIL-R1) and death receptor 4 (DR4), Pan et al., *Science* 276:111-3 (1997), International Patent Application Nos. WO98/32856, WO00/67793, WO99/37684, WO2000/34355, WO99/02653, SEQ ID NO:1); TR7 (also referred to as TRAIL receptor 2 (TRAIL-R2), DR5, and KILLER, Pan et al., *Science* 277:815-8 (1997), Sheridan et al., *Science* 277:818-21 (1997), Chaudhury et al., *Immunity* 7:821-30 (1997), International Patent Application Nos. WO98/46643, WO99/09165, WO99/11791, WO98/41629, WO00/66156, and WO98/35986, SEQ ID NO:3); TR1 (also referred to as Osteoprotegrin (OPG) osteoclastogenesis inhibitory factor (OCIF), TNFRSF11B, and FTHMA-090 (International Patent Application Nos. WO98/12344, WO2000/54651, WO2001/04137, WO66/26217, WO98/07840, WO2000/21554, WO99/53942, and WO2001/03719, SEQ ID NO:5); TR5 (also referred to as TRAIL receptor 3 (TRAIL-R3), decoy receptor 1 (DcR1) and TRID) (Degli-Esposti et al., *J. Exp. Med.* 186:1165-70 (1997), International Patent Application Nos. WO98/30693, WO00/71150, WO99/00423, EP867509, WO98/58062, SEQ ID NO:2); and TR10 (also referred to as TRAIL Receptor 4 (TRAIL-R4), DcR2, and TRUNDD, Pan et al., *FEBS Lett.* 424:41-5 (1998), Degli-Esposti et al., *Immunity* 7:813-20 (1997), International Patent Application Nos. WO98/54202, WO00/73321, WO2000/08155, WO99/03992, WO 2000/34355 and WO9910484, SEQ ID NO:4). TR4 and TR7 contain death domains in their cytoplasmic tails and the triggering of these receptors results in apoptosis. On the other hand, TR1, TR5 and TR10 can inhibit apoptosis induced by the cytotoxic ligand TRAIL in part because of their absent or truncated cytoplasmic death domains, respectively. Each of the publications and patents cited above is hereby incorporated by reference in their entireties, particularly with respect to the nucleotide and amino acid sequences of the TRAIL receptors disclosed therein.

**[0013]** The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of compositions, such as antibodies, that influence the biological activity

of TNF receptors, both normally and in disease states. In particular, there is a need to isolate and characterize antibodies that modulate the biological activities of TRAIL receptors.

### **Summary of the Invention**

[0014] The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a TR4 polypeptide or polypeptide fragment or variant of TR4. In particular, the invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a polypeptide or polypeptide fragment or variant of human TR4 such as that of SEQ ID NO:1. In some embodiments, an antibody of the invention that immunospecifically bind to a TR4 polypeptide, also bind TR7 (e.g., SEQ ID NO:3), but not other proteins, including (TR1, TR5, and TR10 (SEQ ID NOS:5, 2 and 4.)

[0015] The present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TR4 or a fragment or variant thereof. In specific embodiments, the present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with TR4 function or TR4 ligand function or aberrant TR4 or TR4 ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to a TR4 or a fragment or variant thereof. In highly preferred embodiments, the present invention relates to antibody-based methods and compositions for preventing, treating or ameliorating cancers and other hyperproliferative disorders (e.g., leukemia, carcinoma, and lymphoma). Other diseases and disorders which can be treated, prevented or ameliorated with the antibodies of the invention include, but are not limited to, neurodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease, and Huntington's disease), immune disorders (e.g., lupus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, Hashimoto's disease, and immunodeficiency syndrome), inflammatory disorders (e.g., asthma, allergic disorders, and rheumatoid arthritis),

infectious diseases (*e.g.*, AIDS, herpes viral infections, and other viral infections) and proliferative disorders.

**[0016]** In highly preferred embodiments, antibodies of the present invention are used in methods and compositions for preventing, diagnosing, prognosing, treating or ameliorating the following types of cancer: breast cancer, lung cancer, (including non-small cell lung cancer), colon cancer, cancer of the urinary tract, bladder cancer, kidney cancer, pancreatic cancer, liver cancer, stomach cancer, prostate cancer, leukemia, Non-Hodgkin's lymphoma, esophageal cancer, brain cancer, leukemia, ovarian cancer, testicular cancer, melanoma, uterine cancer, cervical cancer, cancer of the larynx, rectal cancer, and cancers of the oral cavity. In specific embodiments, antibodies of the invention are administered in combination with chemotherapeutics such as paclitaxel (Taxol), irinotecan (Camptosar, CPT-11), irinotecan analogs, and gemcitabine (GEMZAR™).

**[0017]** The present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TR4 or a fragment or variant thereof. In specific embodiments, the present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders associated with TR4 function or TR4 ligand function or aberrant TR4 or TR4 ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TR4 or a fragment or variant thereof. In highly preferred embodiments, the present invention relates to antibody-based methods and compositions for detecting, diagnosing, or prognosing cancers and other hyperproliferative disorders (*e.g.*, leukemia, carcinoma, and lymphoma). Other diseases and disorders which can be detected, diagnosed or prognosed with the antibodies of the invention include, but are not limited to, neurodegenerative disorders (*e.g.*, Parkinson's disease, Alzheimer's disease, and Huntington's disease), immune disorders (*e.g.*, lupus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, Hashimoto's disease, and immunodeficiency syndrome), inflammatory disorders (*e.g.*, asthma, allergic disorders, and rheumatoid arthritis), infectious diseases (*e.g.*, AIDS, herpes virus infections, and other viral infections), and proliferative disorders.

**[0018]** Another embodiment of the present invention includes the use of the antibodies of the invention as a diagnostic tool to monitor the expression of TR4 expression on cells.

**[0019]** The present inventors have generated single chain Fv's (scFvs) that immunospecifically bind TR4 polypeptides (e.g., SEQ ID NOs:1). Thus, the invention encompasses these scFvs, listed in Table 1. In addition, the invention encompasses cell lines engineered to express antibodies corresponding to these scFvs which are deposited with the American Type Culture Collection ("ATCC") as of the dates listed in Table 1 and given the ATCC Deposit Numbers identified in Table 1. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

**[0020]** Further, the present invention encompasses the polynucleotides encoding the scFvs, as well as the amino acid sequences encoding the scFvs. Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of any one of the scFvs referred to in Table 1), that immunospecifically bind to TR4 or fragments or variants thereof are also encompassed by the invention, as are nucleic acid molecules that encode these antibodies and/or molecules. In highly preferred embodiments, the present invention encompasses antibodies, or fragments or variants thereof, that bind to the extracellular regions/domains of TR4 or fragments and variants thereof.

**[0021]** The present invention also provides antibodies that bind TR4 polypeptides which are coupled to a detectable label, such as an enzyme, a fluorescent label, a luminescent label, or a bioluminescent label. The present invention also provides antibodies that bind TR4 polypeptides which are coupled to a therapeutic or cytotoxic agent. The present invention also provides antibodies that bind TR4 polypeptides which are coupled to a radioactive material.

**[0022]** The present invention also provides antibodies that bind TR4 polypeptides that act as either TR4 agonists or TR4 antagonists. In specific embodiments, the antibodies of the invention stimulate apoptosis of TR4 expressing cells. In other specific embodiments, the antibodies of the invention inhibit TRAIL binding to TR4. In other specific embodiments, the antibodies of the invention upregulate TR4 expression.

[0023] The present invention also provides antibodies that inhibit apoptosis of TR4 expressing cells. In other specific embodiments, the antibodies of the invention downregulate TR4 expression.

[0024] In further embodiments, the antibodies of the invention have a dissociation constant ( $K_D$ ) of  $10^{-7}$  M or less. In preferred embodiments, the antibodies of the invention have a dissociation constant ( $K_D$ ) of  $10^{-9}$  M or less.

[0025] The present invention further provides antibodies that stimulate apoptosis of TR4 expressing cells better than an equal concentration of TRAIL polypeptide stimulates apoptosis of TR4 expressing cells.

[0026] The present invention further provides antibodies that stimulate apoptosis of TR4 expressing cells equally well in the presence or absence of antibody cross-linking reagents; and/or stimulate apoptosis with equal or greater potency as an equal concentration of TRAIL in the absence of a cross-linking antibody or other cross-linking agent.

[0027] In further embodiments, antibodies of the invention have an off rate ( $k_{off}$ ) of  $10^{-3}$ /sec or less. In preferred embodiments, antibodies of the invention have an off rate ( $k_{off}$ ) of  $10^{-4}$ /sec or less. In other preferred embodiments, antibodies of the invention have an off rate ( $k_{off}$ ) of  $10^{-5}$ /sec or less.

[0028] The present invention also provides for antibodies that preferentially bind TR4 and/or TR7 relative to their ability to bind other proteins (including TR1, TR5 and TR10).

[0029] In certain embodiments, properties of the antibodies of the present invention, as detailed in the Examples below, make the antibodies better therapeutic agents than previously described TR4 binding antibodies.

[0030] The present invention also provides panels of antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) wherein the panel members correspond to one, two, three, four, five, ten, fifteen, twenty, or more different antibodies of the invention (e.g., whole antibodies, Fabs,  $F(ab')_2$  fragments, Fd fragments, disulfide-linked Fvs (sdFvs), anti-idiotypic (anti-Id) antibodies, and scFvs). The present invention further provides mixtures of antibodies, wherein the mixture corresponds to one, two, three, four, five, ten, fifteen, twenty, or more different antibodies of the invention (e.g., whole antibodies, Fabs,  $F(ab')_2$  fragments, Fd fragments, disulfide-linked Fvs (sdFvs), anti-idiotypic (anti-Id) antibodies, and scFvs)). The present invention also provides for compositions comprising, or alternatively consisting of, one, two, three,

four, five, ten, fifteen, twenty, or more antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). A composition of the invention may comprise, or alternatively consist of, one, two, three, four, five, ten, fifteen, twenty, or more amino acid sequences of one or more antibodies or fragments or variants thereof. Alternatively, a composition of the invention may comprise, or alternatively consist of, nucleic acid molecules encoding one or more antibodies of the invention.

**[0031]** The present invention also provides for fusion proteins comprising an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) of the invention, and a heterologous polypeptide (*i.e.*, a polypeptide unrelated to an antibody or antibody domain). Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention. A composition of the present invention may comprise, or alternatively consist of, one, two, three, four, five, ten, fifteen, twenty or more fusion proteins of the invention. Alternatively, a composition of the invention may comprise, or alternatively consist of, nucleic acid molecules encoding one, two, three, four, five, ten, fifteen, twenty or more fusion proteins of the invention.

**[0032]** The present invention also provides for a nucleic acid molecule(s), generally isolated, encoding an antibody (including molecules, such as scFvs, VH domains, or VL domains, that comprise, or alternatively consist of, an antibody fragment or variant thereof) of the invention. The present invention also provides a host cell transformed with a nucleic acid molecule of the invention and progeny thereof. The present invention also provides a method for the production of an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention. The present invention further provides a method of expressing an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention from a nucleic acid molecule. These and other aspects of the invention are described in further detail below.

#### **Brief description of the Figures**

**[0033]** **Figure 1** shows the effect of T1014A04 treatment on SW480 tumor growth in Swiss nu/nu mice with or without Topotecan treatment at 0.3 mg/kg.

**[0034]** **Figure 2** shows the effect of T1014A04 treatment on SW480 tumor growth in Swiss nu/nu mice with or without Topotecan treatment at 0.6 mg/kg.

[0035] **Figure 3** shows the effect of 14G03 treatment on the growth of SW480 tumors in vivo after 28 days with and without Topotecan treatment.

### **Detailed Description of the Invention**

#### **Definitions**

[0036] The term “antibody,” as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. Examples of molecules which are described by the term “antibody” herein include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab’ fragments, F(ab’)<sub>2</sub>, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term “single chain Fv” or “scFv” as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody. Antibodies that immunospecifically bind to TR4 may have cross-reactivity with other antigens. Preferably, antibodies that immunospecifically bind to TR4 do not cross-react with other antigens (e.g., other TRAIL receptors or other members of the Tumor Necrosis Factor Receptor superfamily). Antibodies that immunospecifically bind to TR4 can be identified, for example, by immunoassays or other techniques known to those of skill in the art, *e.g.*, the immunoassays described in the Examples below.

[0037] Antibodies of the invention include, but are not limited to, monoclonal, multispecific, human or chimeric antibodies, single chain antibodies, Fab fragments, F(ab’) fragments, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), intracellularly-made antibodies (*i.e.*, intrabodies), and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub> and IgA<sub>2</sub>) or subclass of immunoglobulin molecule. Preferably, an antibody of the invention comprises, or alternatively consists of, a VH domain, VH CDR, VL domain, or VL CDR having an amino acid sequence of any one of those referred to in Table 1, or a fragment or variant thereof. In a preferred embodiment, the immunoglobulin is an IgG1 isotype. In another preferred embodiment, the immunoglobulin is an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE,



IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

**[0038]** The term "variant" as used herein refers to a polypeptide that possesses a similar or identical function as a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof, but does not necessarily comprise a similar or identical amino acid sequence of a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof, or possess a similar or identical structure of a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof, respectively. A variant having a similar amino acid sequence refers to a polypeptide that satisfies at least one of the following: (a) a polypeptide comprising, or alternatively consisting of, an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of TR4 polypeptide (SEQ ID NO:1), a fragment of , an anti-TR4 antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one or more scFvs referred to in Table 1) described herein; (b) a polypeptide encoded by a nucleotide sequence, the complementary sequence of which hybridizes under stringent conditions to a nucleotide sequence encoding TR4 (SEQ ID NO:1), a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one of those referred to in Table 1), described herein, of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues; and (c) a polypeptide encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%, identical to the nucleotide sequence encoding a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one or more

scFvs referred to in Table 1), described herein. A polypeptide with similar structure to a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody or antibody fragment thereof, described herein refers to a polypeptide that has a similar secondary, tertiary or quaternary structure of a TR4 polypeptide, a fragment of a TR4 polypeptide, an anti-TR4 antibody, or antibody fragment thereof, described herein. The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy.

[0039] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

[0040] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-2268(1990), modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-5877(1993). The BLASTn and BLASTx programs of Altschul, et al. *J. Mol. Biol.* 215:403-410(1990) have incorporated such an algorithm. BLAST nucleotide searches can be performed with the BLASTn program (score = 100, wordlength = 12) to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTx program (score = 50, wordlength = 3) to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. *Nucleic Acids Res.* 25:3589-3402(1997). Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*).

When utilizing BLAST, Gapped BLAST, and PSI-BLAST programs, the default parameters of the respective programs (e.g., BLASTx and BLASTn) can be used. (See <http://www.ncbi.nlm.nih.gov>.)

**[0041]** Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the GCG sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti *Comput. Appl. Biosci.*, 10 :3-5(1994); and FASTA described in Pearson and Lipman *Proc. Natl. Acad. Sci.* 85:2444-8(1988). Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

**[0042]** The term "derivative" as used herein, refers to a variant polypeptide of the invention that comprises, or alternatively consists of, an amino acid sequence of a TR4 polypeptide, a fragment of a TR4 polypeptide, or an antibody of the invention that immunospecifically binds to a TR4 polypeptide, which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a TR4 polypeptide, a fragment of a TR4 polypeptide, an antibody that immunospecifically binds to a TR4 polypeptide which has been modified, *e.g.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a TR4 polypeptide, a fragment of a TR4 polypeptide, or an anti-TR4 antibody, may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a TR4 polypeptide, a fragment of a TR4 polypeptide, or an anti-TR4 antibody, may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a TR4 polypeptide, a fragment of a TR4 polypeptide, or an anti-TR4 antibody, may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as a TR4 polypeptide, a fragment of a TR4 polypeptide, or an anti-TR4 antibody, described herein.

**[0043]** The term "epitopes" as used herein refers to portions of TR4 having antigenic or immunogenic activity in an animal, preferably a mammal. An epitope having

immunogenic activity is a portion of TR4 that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of TR4 to which an antibody immunospecifically binds as determined by any method known in the art, for example, by the immunoassays described herein. Antigenic epitopes need not necessarily be immunogenic.

[0044] The term "fragment" as used herein refers to a polypeptide comprising an amino acid sequence of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 35 amino acid residues, at least 40 amino acid residues, at least 45 amino acid residues, at least 50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues, of the amino acid sequence of TR4, or an anti-TR4 antibody (including molecules such as scFv's, that comprise, or alternatively consist of, antibody fragments or variants thereof).

[0045] The term "fusion protein" as used herein refers to a polypeptide that comprises, or alternatively consists of, an amino acid sequence of an anti-TR4 antibody of the invention and an amino acid sequence of a heterologous polypeptide (*i.e.*, a polypeptide unrelated to an antibody or antibody domain).

[0046] The term "host cell" as used herein refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0047] Antibodies of the present invention are preferably provided in an isolated form, and preferably are substantially purified. By "isolated" is intended an antibody removed from its native environment. Thus, for example, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

### **Antibody Structure**

[0048] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one

"light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

[0049] Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0050] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

[0051] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.,* Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies: small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

[0052] Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are

generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

### **Anti-TR4 Antibodies**

**[0053]** Using phage display technology, the present inventors have identified single chain antibody molecules ("scFvs") that immunospecifically bind to TR4 (or fragments or variants thereof). Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., including VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of any one of those referred to in Table 1), that immunospecifically bind to TR4 (or fragments or variants thereof) are also encompassed by the invention, as are nucleic acid molecules that encode these scFvs, and/or molecules.

**[0054]** In particular, the invention relates to scFvs comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 42-53, preferably SEQ ID NOs:42 and 43 as referred to in Table 1 below. Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., including VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of any one of those referred to in Table 1), that immunospecifically bind to TR4 are also encompassed by the invention, as are nucleic acid molecules that encode these scFvs, and/or molecules (e.g., SEQ ID NOs:54-65).

**[0055]** ScFvs corresponding to SEQ ID NOS:42-53 were selected for their ability bind TR4 polypeptide.

**[0056]** The present invention provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a polypeptide or a polypeptide fragment of TR4. In particular, the invention provides antibodies corresponding to the scFvs referred to in Table 1. Such scFvs may routinely be "converted" to immunoglobulin molecules by inserting, for example, the nucleotide sequences encoding the VH and/or VL domains of the scFv into an expression vector containing the constant domain sequences and engineered to direct the expression of the immunoglobulin molecule, as described in more detail in Example 5 below.

**[0057]** NS0 cell lines that express IgG1 antibodies that comprise the VH and VL domains of scFvs of the invention have been deposited with the American Type Culture Collection ("ATCC") on the dates listed in Table 1 and given the ATCC Deposit Numbers

identified in Table 1. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

**[0058]** Accordingly, in one embodiment, the invention provides antibodies that comprise the VH and VL domains of scFvs of the invention.

**[0059]** In a preferred embodiment, an antibody of the invention is the antibody expressed by cell line NSO  $\alpha$ TRAIL 1985 BU #81 P:15 6/21/01 (See Table 1).

**[0060]** In a preferred embodiment, an antibody of the invention is the antibody expressed by cell line TRAIL (NSO) 14G03 #39 P:14 7/2/01 (See Table 1).

**[0061]** In a preferred embodiment, an antibody of the invention is the antibody expressed by cell line NSO anti-TRAIL 14F08 #28 P:11 (See Table 1).

Table 1: scFvs that Immunospecifically bind to TRAIL Receptors

scFv	scFv protein SEQ ID NO:	scFv DNA SEQ ID NO:	AAs of VH Domain	AAs of VH CDR1	AAs of VH CDR2	AAs of VH CDR3	AAs of VL Domain	AAs of VL CDR1	AAs of VL CDR2	AAs of VL CDR3	Cell Line Expressing antibody	ATCC Deposit Number	ATCC Deposit Date
T1014A04	42	54	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234	NSO $\alpha$ TRAIL 1985 BU #81 P:15 6/21/01	PTA-3571	July 30, 2001
T1014G03	43	55	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234	TRAIL (NSO) 14G03 #39 P:14 7/2/01	PTA-3570	July 30, 2001
T1014A02	44	56	1-116	26-35	50-65	98-105	134-244	156-168	184-190	223-233			
T1014A12	45	57	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1014B01	46	58	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1014B11	47	59	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1014F08	48	60	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234	NSO anti-TRAIL 14F08 #28 P:11	PTA-3675	August 29, 2001
T1014G04	49	61	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1015A02	50	62	1-123	26-37	52-67	100-112	140-250	162-174	190-196	229-239			
T1015A07	51	63	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1015E01	52	64	1-118	26-35	50-66	99-107	135-245	157-170	186-192	225-234			
T1006F07	53	65	1-125	26-35	50-66	99-114	142-249	164-174	190-196	229-238			



[0062] The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a TR4 polypeptide or a fragment, variant, or fusion protein thereof. A TR4 polypeptide includes, but is not limited to, TR4 (SEQ ID NO:1) or the polypeptide encoded by the cDNA in clone HCUDS60 contained in ATCC Deposit 97853 deposited Jan 21, 1997. In some embodiments, antibodies of the present invention may immunospecifically bind to both TR4 as described above and to TR7 (SEQ ID NO:3) or the polypeptide encoded by the cDNA in clone HLYBX88 contained in ATCC Deposit 97920 deposited Mar. 7, 1997. TRAIL receptors may be produced through recombinant expression of nucleic acids encoding the polypeptides of SEQ ID NOS:1-5, (TR4, TR5, TR7, TR10, and TR1; e.g., the cDNAs in the ATCC Deposit Numbers 97853, (TR4) 97798 (TR5, deposited November 20, 1996), 97920 (TR7), or 209040 (TR10, deposited May 15, 1997).

[0063] In one embodiment, the antibodies of the invention preferentially bind TR4 (SEQ ID NO:1), or fragments, variants, or fusion proteins thereof (e.g., the extracellular region of TR4 fused to an Fc domain) relative to their ability to bind other proteins including TR1, TR5, TR7, or TR10 (SEQ ID NOS:5, 2, 3, and 4) or fragments, variants, or fusion proteins thereof. In other preferred embodiments, the antibodies of the invention preferentially bind to TR4 and TR7 (SEQ ID NOS:1 and 3), or fragments or variants thereof relative to their ability to bind other proteins including TR1, TR5 or TR10 (SEQ ID NOS:5, 2 and 4) or fragments, variants, or fusion proteins thereof. In other preferred embodiments, the antibodies of the invention bind TR1 TR4, TR5, TR7 and TR10 (SEQ ID NOS:5, 1, 2, 3, and 4). An antibody's ability to preferentially bind one antigen compared to another antigen may be determined using any method known in the art.

#### **TR4 Polypeptides**

[0064] In certain embodiments of the present invention, the antibodies of the present invention bind TR4 polypeptide, or fragments or variants thereof. The following section describes the TR4 polypeptides, fragments and variants that may be bound by the antibodies of the invention in more detail. The TR4 polypeptides, fragments and variants which may be bound by the antibodies of the invention are also described in International Publication Numbers, for example, WO98/32856 and WO00/67793 which are herein incorporated by reference in their entireties.

**[0065]** In certain embodiments, the antibodies of the present invention immunospecifically bind TR4 polypeptide. An antibody that immunospecifically binds TR4 may, in some embodiments, bind fragments, variants (including species orthologs of TR4), multimers or modified forms of TR4. For example, an antibody immunospecific for TR4 may bind the TR4 moiety of a fusion protein comprising all or a portion of TR4.

**[0066]** TR4 proteins may be found as monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to antibodies that bind TR4 proteins found as monomers or as part of multimers. In specific embodiments, antibodies of the invention bind TR4 monomers, dimers, trimers or tetramers. In additional embodiments, antibodies of the invention bind at least dimers, at least trimers, or at least tetramers containing one or more TR4 polypeptides.

**[0067]** Antibodies of the invention may bind TR4 homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only TR4 proteins of the invention (including TR4 fragments, variants, and fusion proteins, as described herein). These homomers may contain TR4 proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer of the invention is a multimer containing only TR4 proteins having an identical polypeptide sequence. In another specific embodiment, antibodies of the invention bind TR4 homomers containing TR4 proteins having different polypeptide sequences. In specific embodiments, antibodies of the invention bind a TR4 homodimer (e.g., containing TR4 proteins having identical or different polypeptide sequences). In additional embodiments, antibodies of the invention bind at least a homodimer, at least a homotrimer, or at least a homotetramer of TR4.

**[0068]** In specific embodiments antibodies of the present invention bind TR4 homotrimers (e.g., containing TR4 proteins having identical or different polypeptide sequences).

**[0069]** As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing polypeptide sequences that do not correspond to a polypeptide sequences encoded by the TR4 gene) in addition to the TR4 proteins of the invention. In a specific embodiment, antibodies of the invention bind a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the antibodies of the invention bind at least a homodimer, at least a homotrimer, or at least a homotetramer containing one or more TR4 polypeptides.

[0070] In specific embodiments antibodies of the present invention bind a TR4 heterotrimer (e.g., containing 1 or 2 TR4 proteins and 2 or 1, respectively, TR7 proteins).

[0071] Multimers bound by one or more antibodies of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers bound by one or more antibodies of the invention, such as, for example, homodimers or homotrimers, are formed when TR4 proteins contact one another in solution. In another embodiment, heteromultimers bound by one or more antibodies of the invention, such as, for example, heterotrimers or heterotetramers, are formed when proteins of the invention contact antibodies to the TR4 polypeptides (including antibodies to the heterologous polypeptide sequence in a fusion protein) in solution. In other embodiments, multimers bound by one or more antibodies of the invention are formed by covalent associations with and/or between the TR4 proteins of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence recited in SEQ ID NO:1 or the polypeptide encoded by the deposited cDNA clone of ATCC Deposit 97853). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a TR4 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a TR4-Fc fusion protein (as described herein). In another specific example, covalent associations of fusion proteins are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety).

[0072] The multimers that may be bound by one or more antibodies of the invention may be generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked

using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers that may be bound by one or more antibodies of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, proteins that may be bound by one or more antibodies of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer that may be bound by one or more antibodies of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

**[0073]** Alternatively, multimers that may be bound by one or more antibodies of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers that may be bound by one or more antibodies of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer that may be bound by one or more antibodies of the invention are generated by ligating a polynucleotide sequence encoding a TR4 polypeptide to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant TR4 polypeptides which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, two or more TR4

polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple TR4 polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology. In specific embodiments, antibodies of the invention bind proteins comprising multiple TR4 polypeptides separated by peptide linkers.

[0074] Another method for preparing multimer TR4 polypeptides involves use of TR4 polypeptides fused to a leucine zipper or isoleucine polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric TR4 proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble TR4 polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric TR4 is recovered from the culture supernatant using techniques known in the art. In specific embodiments, antibodies of the invention bind TR4-leucine zipper fusion protein monomers and/or TR4-leucine zipper fusion protein multimers.

[0075] Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffer, *Science* 264:667, 1994; Banner et al., *Cell* 73:431, 1993). Thus, trimeric TR4 may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. In specific embodiments, antibodies of the invention bind TR4-leucine zipper fusion protein trimers.

[0076] Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric TR4. In specific embodiments, antibodies of the invention bind TR4- fusion protein monomers and/or TR4 fusion protein trimers.

[0077] Antibodies that bind TR4 receptor polypeptides may bind them as isolated polypeptides or in their naturally occurring state. By "isolated polypeptide" is intended a

polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also, intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the TR4 polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). Thus, antibodies of the present invention may bind recombinantly produced TR4 receptor polypeptides. In a specific embodiment, antibodies of the present invention bind a TR4 receptor expressed on the surface of a cell comprising a polynucleotide encoding amino acids 1 to 468 of SEQ ID NO:1 operably associated with a regulatory sequence that controls gene expression.

[0078] Antibodies of the present invention may bind TR4 polypeptide fragments comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:1, encoded by the cDNA contained in ATCC deposit Number 97853, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in ATCC deposit Number 97853, or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Antibodies of the present invention may bind polypeptide fragments, including, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 23, 24 to 43, 44 to 63, 64 to 83, 84 to 103, 104 to 123, 124 to 143, 144 to 163, 164 to 183, 184 to 203, 204 to 223, 224 to 238, 239 to 264, 265 to 284, 285 to 304, 305 to 324, 325 to 345, 346 to 366, 367 to 387, 388 to 418, 419 to 439, and/or 440 to 468 of SEQ ID NO:1. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Moreover, polypeptide fragments bound by the antibodies of the invention can be at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

[0079] Preferably, antibodies of the present invention bind polypeptide fragments selected from the group: a polypeptide comprising or alternatively, consisting of, the TR4 receptor extracellular domain (predicted to constitute amino acid residues from about 24 to

about 238 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, both TR4 cysteine rich domains (both of which may be found in the protein fragment consisting of amino acid residues from about 131 to about 229 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, the TR4 cysteine rich domain consisting of amino acid residues from about 131 to about 183 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, the TR4 cysteine rich domain consisting of amino acid residues from about 184 to about 229 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, the TR4 receptor transmembrane domain (predicted to constitute amino acid residues from about 239 to about 264 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, fragment of the predicted mature TR4 polypeptide, wherein the fragment has a TR4 functional activity (e.g., antigenic activity or biological activity); a polypeptide comprising or alternatively, consisting of, the TR4 receptor intracellular domain (predicted to constitute amino acid residues from about 265 to about 468 in SEQ ID NO:1); a polypeptide comprising or alternatively, consisting of, the TR4 receptor extracellular and intracellular domains with all or part of the transmembrane domain deleted; a polypeptide comprising, or alternatively consisting of, the TR4 receptor death domain (predicted to constitute amino acid residues from about 379 to about 422 in SEQ ID NO:1); and a polypeptide comprising, or alternatively, consisting of, one, two, three, four or more, epitope bearing portions of the TR4 receptor protein. In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively, consist of, any combination of 1, 2, 3, 4, 5, 6, 7, or all 8 of the above members. The amino acid residues constituting the TR4 receptor extracellular, transmembrane and intracellular domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0080]** It is believed that one or both of the extracellular cysteine rich motifs of TR4 is important for interactions between TR4 and its ligands (e.g., TRAIL). Accordingly, in highly preferred embodiments, antibodies of the present invention bind TR4 polypeptide fragments comprising, or alternatively consisting of amino acid residues 131 to 183, and/or 184 to 229 of SEQ ID NO:1. In another highly preferred embodiment, antibodies of the present invention bind TR4 polypeptides comprising, or alternatively consisting of

both of the extracellular cysteine rich motifs (amino acid residues 131 to 229 of SEQ ID NO:1.) In another preferred embodiment, antibodies of the present invention bind TR4 polypeptides comprising, or alternatively consisting the extracellular soluble domain of TR4 (amino acid residues 24-238 of SEQ ID NO:1.) In highly preferred embodiments, the antibodies of the invention that bind all or a portion of the extracellular soluble domain of TR4 (e.g., one or both cysteine rich domains) prevent TRAIL ligand from binding to TR4. In other highly preferred embodiments, the antibodies of the invention that bind all or a portion of the extracellular soluble domain of TR4 (e.g., one or both cysteine rich domains) agonize the TR4 receptor. In other highly preferred embodiments, the antibodies of the invention that bind all or a portion of the extracellular soluble domain of TR4 (e.g., one or both cysteine rich domains) induce cell death of the cell expressing the TR4 receptor.

**[0081]** Antibodies of the invention may also bind fragments comprising, or alternatively, consisting of structural or functional attributes of TR4. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) TR4. Certain preferred regions are those set out in Table 2 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in (SEQ ID NO:1), such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

**[0082]** The data representing the structural or functional attributes of TR4 set forth in Table 2, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. Column I represents the results of a Garnier-Robson analysis of alpha helical regions; Column II represents the results of a Chou-



Fasman analysis of alpha helical regions; Column III represents the results of a Garnier Robson analysis of beta sheet regions; Column IV represents the results of a Chou-Fasman analysis of beta sheet regions; Column V represents the results of a Garnier Robson analysis of turn regions; Column VI represents the results of a Chou-Fasman analysis of turn regions; Column VII represents the results of a Garnier Robson analysis of coil regions; Column VIII represents a Kyte-Doolittle hydrophilicity plot; Column IX represents the results of an Eisenberg analysis of alpha amphipathic regions; Column X represents the results of an Eisenberg analysis of beta amphipathic regions; Column XI represents the results of a Karplus-Schultz analysis of flexible regions; Column XII represents the Jameson-Wolf antigenic index score; and Column XIII represents the Emini surface probability plot.

[0083] In a preferred embodiment, the data presented in columns VIII, XII, and XIII of Table 2 can be used to determine regions of TR4 which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, XII, and/or XIII by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0084] The above-mentioned preferred regions set out in Table 2 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in SEQ ID NO:1. As set out in Table 2, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Jameson-Wolf regions of high antigenic index and Emini surface-forming regions. Among preferred polypeptide fragments bound by one or more antibodies of the invention are those that comprise regions of TR4 that combine several structural features, such as several (e.g., 1, 2, 3, or 4) of the same or different region features set out above and in Table 2.

Table 2

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Met	1	.	.	B	.	.	.	.	0.12	.	.	.	-0.10	0.90
Ala	2	.	.	.	.	.	.	C	-0.08	*	*	.	0.25	1.08
Pro	3	.	.	.	.	.	.	C	0.42	*	*	.	0.10	0.86
Pro	4	.	.	.	.	.	T	C	-0.04	*	*	.	1.05	1.69
Pro	5	A	.	.	.	.	T	.	0.31	.	*	F	1.00	1.24
Ala	6	A	.	.	.	.	T	.	0.10	.	*	F	1.00	1.10
Arg	7	A	.	.	.	.	T	.	0.34	.	*	.	0.10	0.58
Val	8	.	.	B	B	.	.	.	-0.03	.	*	.	-0.30	0.37
His	9	.	.	B	B	.	.	.	-0.52	.	*	.	-0.30	0.37
Leu	10	.	.	B	B	.	.	.	-1.12	.	*	.	-0.60	0.17
Gly	11	.	.	B	B	.	.	.	-1.12	.	*	.	-0.60	0.18
Ala	12	.	.	B	B	.	.	.	-2.09	.	*	.	-0.60	0.14
Phe	13	.	.	B	B	.	.	.	-1.54	.	*	.	-0.60	0.12
Leu	14	.	.	B	B	.	.	.	-1.72	.	.	.	-0.60	0.18
Ala	15	.	.	B	B	.	.	.	-0.91	.	.	.	-0.60	0.27
Val	16	.	.	B	B	.	.	.	-0.78	.	.	.	-0.60	0.51
Thr	17	.	.	B	B	.	.	.	-0.53	.	.	F	-0.45	0.95
Pro	18	.	.	.	B	.	.	C	-0.13	.	.	F	0.05	0.93
Asn	19	.	.	.	.	.	T	C	0.09	.	.	F	0.60	1.69
Pro	20	.	.	.	.	.	T	C	0.09	.	.	F	0.60	1.18
Gly	21	.	.	.	.	T	T	.	0.64	.	.	F	0.65	0.77
Ser	22	.	.	.	.	.	T	C	0.61	.	.	F	0.45	0.64
Ala	23	.	.	.	.	.	.	C	0.51	.	.	F	0.25	0.41
Ala	24	.	.	.	.	.	T	C	0.51	.	.	F	0.45	0.60
Ser	25	.	.	B	.	.	T	.	0.13	.	.	F	0.85	0.78
Gly	26	A	.	.	.	.	T	.	-0.11	.	.	F	0.85	0.78
Thr	27	A	.	.	.	.	T	.	-0.40	.	.	F	0.85	0.78
Glu	28	A	A	.	.	.	.	.	-0.40	.	.	F	0.45	0.58
Ala	29	A	A	.	.	.	.	.	-0.12	.	.	.	0.30	0.60
Ala	30	A	A	.	.	.	.	.	-0.03	.	.	.	0.30	0.60
Ala	31	A	A	.	.	.	.	.	0.01	.	.	.	0.30	0.53
Ala	32	A	A	.	.	.	.	.	0.37	.	.	.	-0.30	0.71
Thr	33	A	.	.	.	.	T	.	-0.49	*	.	F	1.00	1.40
Pro	34	A	.	.	.	.	T	.	-0.19	.	.	F	1.00	1.03
Ser	35	.	.	B	.	.	T	.	0.06	.	.	F	0.40	1.07
Lys	36	.	.	B	.	.	T	.	0.34	.	.	F	0.25	0.73
Val	37	.	.	B	B	.	.	.	0.63	.	.	F	-0.15	0.64
Trp	38	.	.	B	B	.	.	.	0.36	.	.	F	-0.15	0.64
Gly	39	.	.	B	B	.	.	.	0.22	*	*	F	-0.15	0.32
Ser	40	.	.	.	.	.	.	C	0.63	*	*	F	-0.05	0.43
Ser	41	.	.	.	.	.	T	C	-0.30	*	*	F	0.45	0.80
Ala	42	.	.	.	.	.	T	C	0.56	*	*	F	1.05	0.57
Gly	43	.	.	.	.	.	T	C	0.63	*	*	F	1.35	0.73
Arg	44	.	.	B	.	.	T	.	1.09	*	*	F	1.49	0.84
Ile	45	.	.	B	.	.	.	.	1.04	*	*	F	1.78	1.63
Glu	46	.	.	B	.	.	.	.	1.00	*	*	F	2.12	1.63
Pro	47	.	.	B	.	.	T	.	1.24	*	*	F	2.51	0.83
Arg	48	.	.	.	.	T	T	.	1.70	*	*	F	3.40	1.17
Gly	49	.	.	.	.	T	T	.	1.24	*	*	F	3.06	1.32
Gly	50	.	.	.	.	T	T	.	1.54	*	*	F	2.57	0.84

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gly	51	.	.	.	.	.	T	C	0.73	*	*	F	2.03	0.44
Arg	52	.	.	.	.	.	T	C	0.73	*	*	F	1.39	0.36
Gly	53	.	.	B	.	.	T	.	0.31	*	*	F	0.85	0.57
Ala	54	.	.	B	.	.	T	.	0.36	.	*	F	0.85	0.83
Leu	55	.	.	B	.	.	.	.	0.10	.	*	F	0.65	0.57
Pro	56	.	.	B	.	.	.	.	0.10	.	*	F	-0.25	0.57
Thr	57	.	.	B	.	.	.	.	-0.01	.	*	F	-0.25	0.55
Ser	58	.	.	B	.	.	T	.	0.30	.	.	F	0.10	1.16
Met	59	.	.	B	.	.	T	.	0.54	.	.	F	0.40	1.02
Gly	60	.	.	B	.	.	T	.	1.14	.	.	F	0.25	0.70
Gln	61	.	.	.	.	T	T	.	1.06	.	.	F	0.65	0.81
His	62	.	.	.	.	.	.	C	0.78	.	*	F	0.40	1.10
Gly	63	.	.	.	.	.	T	C	1.19	.	*	F	0.60	1.12
Pro	64	.	.	.	.	.	T	C	1.20	.	*	F	1.20	1.27
Ser	65	.	.	.	.	.	T	C	1.66	.	*	F	1.05	0.94
Ala	66	.	.	B	.	.	T	.	1.07	.	*	F	1.30	1.86
Arg	67	.	.	B	.	.	.	.	0.76	*	*	.	1.29	1.22
Ala	68	.	.	B	.	.	.	.	1.21	*	*	.	1.48	0.90
Arg	69	.	.	B	.	.	T	.	0.83	.	*	.	2.17	1.74
Ala	70	.	.	B	.	.	T	.	0.92	.	*	F	2.51	0.90
Gly	71	.	.	.	.	T	T	.	1.17	.	*	F	3.40	1.37
Arg	72	.	.	.	.	.	T	C	0.84	.	*	F	2.71	0.69
Ala	73	.	.	.	.	.	T	C	1.54	*	.	F	2.48	1.06
Pro	74	.	.	.	.	.	T	C	1.22	*	.	F	2.70	2.10
Gly	75	.	.	.	.	.	T	C	1.22	*	.	F	2.62	1.66
Pro	76	.	.	.	.	.	T	C	1.68	*	*	F	2.24	1.66
Arg	77	.	.	.	.	.	.	C	1.57	*	.	F	2.60	2.10
Pro	78	.	A	B	.	.	.	.	1.57	*	.	F	1.94	3.68
Ala	79	.	A	B	.	.	.	.	1.48	*	.	F	1.68	2.40
Arg	80	.	A	B	.	.	.	.	1.61	*	*	F	1.42	1.64
Glu	81	.	A	B	.	.	.	.	1.93	*	*	F	1.16	1.64
Ala	82	A	A	.	.	.	.	.	1.01	*	*	F	0.90	3.19
Ser	83	A	.	.	.	.	T	.	1.33	*	*	F	1.30	1.34
Pro	84	A	.	.	.	.	T	.	1.07	*	*	F	1.30	1.52
Arg	85	A	.	.	.	.	T	.	0.92	*	*	F	1.00	1.12
Leu	86	A	.	.	.	.	T	.	0.97	.	*	.	0.85	1.13
Arg	87	A	.	.	B	.	.	.	1.24	.	*	.	0.75	1.46
Val	88	A	.	.	B	.	.	.	0.84	*	*	.	0.75	1.08
His	89	A	.	.	B	.	.	.	1.10	.	*	.	-0.15	1.13
Lys	90	A	.	.	B	.	.	.	0.29	*	*	F	0.90	1.16
Thr	91	.	.	B	B	.	.	.	0.24	*	*	F	0.00	1.35
Phe	92	.	.	B	B	.	.	.	-0.72	*	*	.	-0.30	0.74
Lys	93	.	.	B	B	.	.	.	-0.72	*	*	.	-0.30	0.27
Phe	94	.	.	B	B	.	.	.	-1.03	*	.	.	-0.60	0.14
Val	95	.	.	B	B	.	.	.	-1.93	*	.	.	-0.60	0.16
Val	96	.	.	B	B	.	.	.	-2.43	.	*	.	-0.60	0.06
Val	97	.	.	B	B	.	.	.	-2.54	.	*	.	-0.60	0.06
Gly	98	.	.	B	B	.	.	.	-2.59	.	*	.	-0.60	0.06
Val	99	.	.	B	B	.	.	.	-2.74	.	.	.	-0.60	0.15
Leu	100	.	.	B	B	.	.	.	-2.74	*	.	.	-0.60	0.15

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Leu	101	.	.	B	B	.	.	.	-2.10	*	.	.	-0.60	0.11
Gln	102	.	.	B	B	.	.	.	-1.54	*	.	.	-0.60	0.23
Val	103	.	.	B	B	.	.	.	-1.50	.	.	.	-0.60	0.37
Val	104	.	.	B	.	.	T	.	-1.23	.	.	.	-0.20	0.61
Pro	105	.	.	B	.	.	T	.	-1.01	*	.	F	0.25	0.35
Ser	106	A	.	.	.	.	T	.	-0.51	*	.	F	-0.05	0.48
Ser	107	A	.	.	.	.	T	.	-1.40	*	*	F	0.25	0.94
Ala	108	A	.	.	.	.	.	.	-0.50	.	*	F	0.05	0.43
Ala	109	A	.	.	.	.	.	.	-0.46	.	*	.	0.50	0.63
Thr	110	A	.	.	.	.	.	.	-0.28	.	*	.	-0.10	0.39
Ile	111	A	.	.	.	.	.	.	0.02	.	*	.	-0.10	0.53
Lys	112	.	.	B	.	.	.	.	0.32	.	*	.	0.50	0.87
Leu	113	.	.	B	.	.	.	.	0.61	.	*	F	1.05	1.04
His	114	.	.	B	.	.	.	.	0.31	.	*	F	1.30	1.99
Asp	115	.	.	.	.	.	T	C	0.28	*	*	F	1.80	0.70
Gln	116	.	.	.	.	T	T	.	0.86	.	*	F	1.65	0.84
Ser	117	.	.	.	.	T	T	.	0.81	.	.	F	2.50	0.89
Ile	118	.	.	.	.	T	T	.	1.62	.	.	F	2.25	0.92
Gly	119	.	.	.	.	.	.	C	1.37	.	.	F	1.00	0.92
Thr	120	.	.	.	.	.	.	C	1.37	.	.	F	0.45	0.72
Gln	121	.	.	B	.	.	.	C	1.33	.	.	F	0.65	1.79
Gln	122	.	.	B	.	.	.	.	1.33	.	.	F	0.20	2.46
Trp	123	.	.	B	.	.	.	.	2.01	.	.	.	0.05	2.28
Glu	124	.	.	.	.	.	.	C	1.54	.	.	.	0.25	2.04
His	125	.	.	.	.	.	.	C	1.51	.	.	.	0.10	0.97
Ser	126	.	.	.	.	.	T	C	1.51	.	.	F	0.45	0.91
Pro	127	.	.	.	.	T	T	.	0.70	.	.	F	1.55	0.91
Leu	128	.	.	.	.	T	T	.	0.32	.	.	F	0.65	0.55
Gly	129	.	.	.	.	T	T	.	0.11	.	.	F	0.65	0.22
Glu	130	.	.	.	.	T	.	.	-0.07	.	.	F	0.45	0.22
Leu	131	.	.	B	.	.	.	.	-0.11	*	.	.	0.18	0.42
Cys	132	.	.	B	.	.	.	.	-0.20	*	.	F	1.21	0.42
Pro	133	.	.	B	.	.	T	.	0.58	*	*	F	1.69	0.32
Pro	134	.	.	.	.	T	T	.	1.03	.	*	F	1.47	0.53
Gly	135	.	.	.	.	T	T	.	0.73	.	*	F	2.80	1.94
Ser	136	.	.	.	.	.	T	C	1.54	*	.	F	2.32	1.68
His	137	.	.	.	.	.	.	C	2.32	*	.	F	2.48	1.88
Arg	138	.	.	B	.	.	.	.	2.32	*	.	F	2.34	3.72
Ser	139	.	.	B	.	.	.	.	2.19	*	.	F	2.40	4.29
Glu	140	.	.	.	.	T	.	.	1.94	*	.	F	2.86	3.12
Arg	141	.	.	.	.	T	T	.	1.58	*	.	F	3.40	1.61
Pro	142	.	.	.	.	T	T	.	1.61	.	*	F	2.91	0.64
Gly	143	.	.	.	.	T	T	.	1.61	.	*	F	2.57	0.60
Ala	144	.	.	.	.	T	T	.	1.24	.	*	.	2.08	0.60
Cys	145	.	.	.	.	T	.	.	0.93	.	*	.	1.41	0.21
Asn	146	.	.	B	.	.	.	.	0.82	.	*	.	0.84	0.30
Arg	147	.	.	B	.	.	.	.	0.69	*	.	.	1.01	0.52
Cys	148	.	.	B	.	.	T	.	0.18	*	.	F	1.83	0.96
Thr	149	.	.	B	.	.	T	.	0.42	*	.	F	1.70	0.44
Glu	150	.	.	B	.	.	T	.	0.84	*	.	F	1.53	0.22

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gly	151	.	.	B	.	.	T	.	0.53	*	.	F	0.76	0.65
Val	152	.	.	B	B	.	.	.	0.42	.	*	F	0.19	0.65
Gly	153	.	.	B	B	.	.	.	0.50	.	.	.	-0.13	0.61
Tyr	154	.	.	B	B	.	.	.	0.51	.	.	.	-0.60	0.62
Thr	155	.	.	B	B	.	.	.	0.51	.	.	F	-0.30	1.12
Asn	156	.	.	.	B	.	.	C	0.86	.	.	F	0.20	1.81
Ala	157	.	.	.	.	T	T	.	0.90	.	.	F	0.80	1.86
Ser	158	.	.	.	.	T	T	.	0.54	.	.	F	0.80	1.06
Asn	159	.	.	.	.	T	T	.	0.20	.	.	F	0.35	0.57
Asn	160	.	.	.	.	T	T	.	-0.16	*	.	F	0.35	0.57
Leu	161	.	A	B	.	.	.	.	-0.97	*	.	.	-0.60	0.23
Phe	162	.	A	B	.	.	.	.	-0.59	.	.	.	-0.60	0.12
Ala	163	.	A	B	.	.	.	.	-0.96	.	.	.	-0.60	0.11
Cys	164	.	A	B	.	.	.	.	-1.27	*	.	.	-0.60	0.07
Leu	165	.	.	B	.	.	T	.	-1.86	.	.	.	-0.20	0.12
Pro	166	.	.	B	.	.	T	.	-1.71	*	.	.	-0.20	0.12
Cys	167	.	.	.	.	T	T	.	-0.97	*	.	.	0.20	0.12
Thr	168	A	.	.	.	.	T	.	-0.68	.	.	.	0.10	0.30
Ala	169	A	.	.	.	.	.	.	-0.01	.	.	.	0.50	0.26
Cys	170	A	.	.	.	.	T	.	0.80	.	.	.	0.70	0.80
Lys	171	A	.	.	.	.	T	.	1.01	.	.	F	1.15	0.96
Ser	172	A	.	.	.	.	T	.	1.68	.	*	F	1.30	1.65
Asp	173	A	.	.	.	.	T	.	2.10	.	*	F	1.30	5.33
Glu	174	A	A	.	.	.	.	.	2.39	.	*	F	0.90	5.22
Glu	175	A	A	.	.	.	.	.	2.84	.	*	F	1.24	5.22
Glu	176	A	A	.	.	.	.	.	2.13	.	*	F	1.58	4.83
Arg	177	.	A	.	.	T	.	.	2.12	.	.	F	2.32	1.50
Ser	178	.	.	.	.	.	T	C	1.81	.	.	F	2.86	1.25
Pro	179	.	.	.	.	T	T	.	1.50	*	.	F	3.40	1.04
Cys	180	.	.	.	.	T	T	.	1.61	*	.	F	2.61	0.77
Thr	181	.	.	.	.	T	T	.	1.61	*	.	F	2.67	1.12
Thr	182	.	.	.	.	T	.	.	1.19	*	*	F	2.38	1.16
Thr	183	.	.	.	.	T	T	.	0.90	.	.	F	2.49	3.13
Arg	184	.	.	.	.	T	T	.	0.44	.	.	F	2.40	2.19
Asn	185	.	.	.	.	T	T	.	1.11	.	.	F	2.50	0.81
Thr	186	.	.	.	.	T	T	.	0.76	*	.	F	2.25	0.98
Ala	187	.	.	.	.	T	.	.	1.11	*	.	.	1.65	0.27
Cys	188	.	.	.	.	T	.	.	1.21	*	.	.	1.40	0.33
Gln	189	.	.	B	.	.	.	.	0.76	*	.	.	0.75	0.36
Cys	190	.	.	B	.	.	.	.	0.44	.	.	.	0.50	0.35
Lys	191	.	.	B	.	.	T	.	0.06	.	*	F	0.85	0.94
Pro	192	.	.	.	.	T	T	.	0.76	.	.	F	0.65	0.47
Gly	193	.	.	.	.	T	T	.	1.42	.	*	F	1.74	1.72
Thr	194	.	.	B	.	.	T	.	1.42	.	*	F	1.68	1.38
Phe	195	.	.	B	.	.	.	.	2.09	.	*	F	1.82	1.49
Arg	196	.	.	.	.	T	.	.	1.74	.	*	F	2.56	2.42
Asn	197	.	.	.	.	T	T	.	1.37	.	*	F	3.40	2.25
Asp	198	.	.	.	.	T	T	.	1.71	.	*	F	3.06	2.63
Asn	199	.	.	.	.	.	T	C	1.42	.	*	F	2.52	2.32
Ser	200	A	.	.	.	.	T	.	1.46	.	*	F	1.98	1.43

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ala	201	A	.	.	.	.	.	.	1.46	.	*	.	1.14	0.46
Glu	202	A	.	.	.	.	.	.	1.50	*	.	.	0.80	0.56
Met	203	A	.	.	.	.	.	.	0.83	*	.	.	1.11	0.83
Cys	204	A	.	.	.	.	T	.	0.53	*	.	.	1.62	0.44
Arg	205	.	.	.	.	T	T	.	0.52	*	.	.	2.33	0.34
Lys	206	.	.	.	.	T	T	.	0.77	*	.	F	2.49	0.50
Cys	207	.	.	.	.	T	T	.	0.10	*	.	F	3.10	0.92
Ser	208	.	.	.	.	T	.	.	0.49	*	*	F	2.59	0.25
Thr	209	.	.	.	.	T	.	.	1.27	*	*	F	1.98	0.19
Gly	210	.	.	.	.	T	.	.	0.81	*	.	F	1.67	0.71
Cys	211	.	.	B	.	.	T	.	0.17	*	*	F	1.16	0.53
Pro	212	.	.	.	.	T	T	.	-0.02	*	*	F	1.25	0.36
Arg	213	.	.	.	.	T	T	.	0.32	*	*	F	0.65	0.27
Gly	214	.	.	B	.	.	T	.	-0.22	*	*	.	0.85	1.01
Met	215	.	.	B	B	.	.	.	0.17	*	*	.	0.30	0.48
Val	216	.	.	B	B	.	.	.	0.83	*	*	.	0.79	0.49
Lys	217	.	.	B	B	.	.	.	0.38	*	*	.	0.98	0.83
Val	218	.	.	B	B	.	.	.	-0.04	*	*	F	1.32	0.45
Lys	219	.	.	B	B	.	.	.	0.09	.	*	F	1.51	0.88
Asp	220	.	.	B	.	.	.	.	0.40	.	*	F	1.90	0.68
Cys	221	.	.	B	.	.	.	.	0.96	.	*	F	0.81	0.96
Thr	222	.	.	.	.	.	T	C	0.91	.	*	F	1.62	0.65
Pro	223	.	.	.	.	T	T	.	0.88	.	*	F	1.63	0.65
Trp	224	.	.	.	.	T	T	.	0.83	.	*	F	0.54	0.84
Ser	225	A	.	.	.	.	T	.	0.17	.	.	F	1.00	1.01
Asp	226	A	A	.	.	.	.	.	-0.02	.	.	F	0.45	0.35
Ile	227	A	A	.	.	.	.	.	0.26	*	.	.	-0.30	0.25
Glu	228	A	A	.	.	.	.	.	0.51	*	.	.	0.30	0.25
Cys	229	.	A	B	.	.	.	.	0.80	*	.	.	0.60	0.30
Val	230	A	A	.	.	.	.	.	0.80	*	*	.	0.60	0.74
His	231	A	A	.	.	.	.	.	0.46	*	*	.	0.60	0.58
Lys	232	A	A	.	.	.	.	.	1.34	*	.	F	0.60	1.06
Glu	233	.	A	.	.	T	.	.	1.00	*	.	F	1.30	2.30
Ser	234	.	.	.	.	T	T	.	1.63	*	.	F	1.70	1.68
Gly	235	.	.	.	.	T	T	.	2.49	*	.	F	1.70	1.14
Asn	236	.	.	.	.	T	T	.	1.63	*	.	F	1.40	1.06
Gly	237	.	.	.	.	.	T	C	1.30	*	.	F	0.45	0.55
His	238	.	.	.	B	.	.	C	0.44	.	.	.	-0.40	0.59
Asn	239	.	.	.	B	.	.	C	-0.14	.	.	.	-0.40	0.27
Ile	240	.	.	B	B	.	.	.	-0.61	.	.	.	-0.60	0.19
Trp	241	.	.	B	B	.	.	.	-1.47	.	.	.	-0.60	0.12
Val	242	.	.	B	B	.	.	.	-1.98	.	.	.	-0.60	0.05
Ile	243	.	.	B	B	.	.	.	-2.26	.	.	.	-0.60	0.06
Leu	244	.	.	B	B	.	.	.	-3.07	.	.	.	-0.60	0.08
Val	245	.	.	B	B	.	.	.	-3.03	.	.	.	-0.60	0.09
Val	246	.	.	B	B	.	.	.	-3.60	.	.	.	-0.60	0.09
Thr	247	.	.	B	B	.	.	.	-2.96	.	.	.	-0.60	0.08
Leu	248	.	.	B	B	.	.	.	-2.88	.	.	.	-0.60	0.17
Val	249	.	.	B	B	.	.	.	-2.88	.	*	.	-0.60	0.19
Val	250	.	.	B	B	.	.	.	-2.83	.	.	.	-0.60	0.11

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pro	251	.	.	B	B	.	.	.	-2.83	.	.	.	-0.60	0.11
Leu	252	.	.	B	B	.	.	.	-3.11	.	.	.	-0.60	0.11
Leu	253	A	.	.	B	.	.	.	-3.16	.	.	.	-0.60	0.15
Leu	254	A	.	.	B	.	.	.	-3.11	.	.	.	-0.60	0.07
Val	255	A	.	.	B	.	.	.	-3.14	.	.	.	-0.60	0.07
Ala	256	A	.	.	B	.	.	.	-3.79	.	.	.	-0.60	0.06
Val	257	.	.	B	B	.	.	.	-3.64	.	.	.	-0.60	0.05
Leu	258	.	.	B	B	.	.	.	-3.50	.	.	.	-0.60	0.04
Ile	259	.	.	B	B	.	.	.	-3.36	.	.	.	-0.60	0.02
Val	260	.	.	B	B	.	.	.	-3.39	.	.	.	-0.60	0.02
Cys	261	.	.	B	B	.	.	.	-3.14	.	.	.	-0.60	0.01
Cys	262	.	.	B	B	.	.	.	-2.59	.	.	.	-0.60	0.02
Cys	263	.	.	B	B	.	.	.	-2.12	.	.	.	-0.60	0.03
Ile	264	.	.	B	B	.	.	.	-1.90	.	.	.	-0.60	0.06
Gly	265	.	.	.	.	T	T	.	-1.39	.	.	F	0.35	0.06
Ser	266	.	.	.	.	T	T	.	-1.07	.	.	F	0.35	0.11
Gly	267	.	.	.	.	T	T	.	-0.40	.	.	F	0.65	0.16
Cys	268	.	.	.	.	T	T	.	0.06	.	.	F	1.25	0.27
Gly	269	.	.	.	.	T	.	.	0.99	.	*	F	1.39	0.31
Gly	270	.	.	.	.	T	.	.	0.67	.	.	F	2.03	0.62
Asp	271	.	.	.	.	.	T	C	0.37	.	.	F	2.37	0.62
Pro	272	.	.	.	.	T	T	.	0.71	*	*	F	2.91	0.62
Lys	273	.	.	.	.	T	T	.	1.49	*	*	F	3.40	1.05
Cys	274	.	.	B	.	.	T	.	0.98	*	*	.	2.51	1.23
Met	275	.	.	B	B	.	.	.	0.66	*	*	.	1.62	0.59
Asp	276	.	.	B	B	.	.	.	-0.04	*	*	.	1.28	0.16
Arg	277	.	.	B	B	.	.	.	-0.12	.	*	.	0.04	0.26
Val	278	.	.	B	B	.	.	.	-0.06	.	*	.	-0.60	0.27
Cys	279	.	.	B	B	.	.	.	-0.20	.	.	.	0.30	0.32
Phe	280	.	.	B	B	.	.	.	0.06	.	*	.	-0.60	0.13
Trp	281	.	.	B	B	.	.	.	-0.76	.	.	.	-0.60	0.18
Arg	282	.	.	B	B	.	.	.	-1.68	.	.	.	-0.60	0.28
Leu	283	.	.	B	B	.	.	.	-0.71	.	.	.	-0.60	0.26
Gly	284	.	.	.	B	T	.	.	-0.39	.	*	.	-0.20	0.49
Leu	285	.	.	.	B	.	.	C	0.10	.	*	.	0.50	0.25
Leu	286	.	.	.	B	.	.	C	0.04	.	*	.	0.20	0.46
Arg	287	.	.	.	B	.	.	C	-0.66	.	.	F	0.65	0.46
Gly	288	.	.	.	.	.	T	C	0.16	.	.	F	1.35	0.57
Pro	289	.	.	.	.	.	T	C	0.50	.	*	F	2.70	1.19
Gly	290	.	.	.	.	.	T	C	1.31	*	*	F	3.00	1.01
Ala	291	A	.	.	.	.	T	.	1.53	.	*	F	2.50	1.65
Glu	292	A	.	.	.	.	.	.	1.39	.	.	F	2.00	1.08
Asp	293	A	.	.	.	.	.	.	1.73	.	.	F	1.70	1.48
Asn	294	A	.	.	.	.	T	.	1.94	.	*	.	1.45	2.36
Ala	295	A	.	.	.	.	T	.	1.40	.	.	.	1.15	2.36
His	296	A	.	.	.	.	T	.	1.18	*	.	.	1.00	0.99
Asn	297	A	.	.	.	.	T	.	0.88	.	.	.	0.10	0.51
Glu	298	A	.	.	.	.	.	.	0.88	*	.	.	-0.10	0.67
Ile	299	A	.	.	.	.	.	.	0.29	*	*	.	-0.10	0.80
Leu	300	A	.	.	.	.	.	.	0.88	*	*	.	-0.10	0.50

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ser	301	A	.	.	.	.	.	.	0.61	*	.	F	0.65	0.48
Asn	302	A	.	.	.	.	T	.	-0.20	*	.	F	0.25	0.92
Ala	303	A	.	.	.	.	T	.	-0.50	*	.	F	0.25	0.92
Asp	304	A	.	.	.	.	T	.	0.08	*	.	F	0.85	0.92
Ser	305	.	.	.	.	.	T	C	0.19	*	.	F	1.05	0.83
Leu	306	.	.	.	B	.	.	C	-0.37	*	.	F	0.05	0.71
Ser	307	.	.	B	B	.	.	.	-0.67	*	.	F	-0.15	0.31
Thr	308	.	.	B	B	.	.	.	-0.08	*	.	.	-0.60	0.31
Phe	309	.	.	B	B	.	.	.	-0.08	*	.	.	-0.30	0.66
Val	310	A	.	.	B	.	.	.	0.22	.	.	F	-0.15	0.85
Ser	311	A	A	.	.	.	.	.	0.43	.	.	F	0.00	1.03
Glu	312	A	A	.	.	.	.	.	0.73	.	.	F	0.00	1.17
Gln	313	A	A	.	.	.	.	.	0.74	.	.	F	0.90	2.73
Gln	314	A	A	.	.	.	.	.	1.44	.	.	F	0.90	2.73
Met	315	A	A	.	.	.	.	.	2.30	.	.	F	0.90	2.73
Glu	316	A	A	.	.	.	.	.	2.39	.	.	F	0.90	2.73
Ser	317	A	A	.	.	.	.	.	1.80	.	*	F	0.90	2.44
Gln	318	A	A	.	.	.	.	.	1.80	.	*	F	0.90	2.49
Glu	319	A	A	.	.	.	.	.	0.99	.	*	F	0.90	2.40
Pro	320	A	A	.	.	.	.	.	1.28	.	*	F	0.90	1.48
Ala	321	A	A	.	.	.	.	.	0.93	.	.	F	0.60	1.23
Asp	322	A	A	.	B	.	.	.	0.38	.	.	F	0.45	0.70
Leu	323	A	A	.	B	.	.	.	0.07	.	.	F	-0.15	0.34
Thr	324	.	A	B	B	.	.	.	-0.79	.	.	F	-0.15	0.48
Gly	325	.	A	B	B	.	.	.	-0.58	.	.	.	-0.30	0.21
Val	326	.	.	B	B	.	.	.	-0.29	.	.	.	-0.60	0.45
Thr	327	.	.	B	B	.	.	.	-0.50	.	.	.	-0.60	0.42
Val	328	.	.	B	B	.	.	.	-0.03	.	*	F	-0.17	0.65
Gln	329	.	.	B	B	.	.	.	0.28	.	*	F	0.11	0.87
Ser	330	.	.	.	.	.	T	C	0.03	.	*	F	2.04	1.05
Pro	331	.	.	.	.	.	T	C	0.89	.	*	F	2.32	1.42
Gly	332	.	.	.	.	T	T	.	0.53	.	*	F	2.80	1.42
Glu	333	A	.	.	.	.	T	.	0.58	.	*	F	1.97	0.57
Ala	334	.	.	B	.	.	.	.	-0.23	.	*	.	0.74	0.30
Gln	335	.	.	B	.	.	.	.	-0.28	.	.	.	0.46	0.25
Cys	336	.	.	B	.	.	.	.	-0.28	.	.	.	0.18	0.14
Leu	337	.	.	B	.	.	.	.	-0.52	.	*	.	-0.40	0.22
Leu	338	.	.	B	.	.	.	.	-0.52	.	*	.	-0.40	0.13
Gly	339	.	A	.	.	.	.	C	-0.52	.	*	F	0.05	0.42
Pro	340	A	A	.	.	.	.	.	-0.52	.	*	F	-0.15	0.51
Ala	341	A	A	.	.	.	.	.	-0.20	.	*	F	0.60	1.07
Glu	342	A	A	.	.	.	.	.	0.31	.	*	F	0.90	1.07
Ala	343	A	A	.	.	.	.	.	1.12	*	*	F	0.75	0.93
Glu	344	A	A	.	.	.	.	.	1.58	.	*	F	0.90	1.60
Gly	345	A	A	.	.	.	.	.	1.90	.	*	F	0.90	1.80
Ser	346	A	.	.	.	.	T	.	2.60	.	*	F	1.30	3.50
Gln	347	A	.	.	.	.	T	.	1.79	.	*	F	1.30	3.96
Arg	348	A	.	.	.	.	T	.	1.57	.	*	F	1.30	3.30
Arg	349	.	.	B	.	.	T	.	0.71	.	*	F	1.30	2.03
Arg	350	.	.	B	B	.	.	.	0.84	.	*	F	0.75	0.87



Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Leu	351	.	.	B	B	.	.	.	0.56	.	*	.	0.60	0.69
Leu	352	.	.	B	B	.	.	.	0.56	.	*	.	0.30	0.35
Val	353	.	.	B	B	.	.	.	0.10	*	*	.	-0.30	0.29
Pro	354	.	.	B	.	.	T	.	-0.60	*	.	.	-0.20	0.35
Ala	355	.	.	.	.	T	T	.	-0.71	.	*	.	0.50	0.43
Asn	356	.	.	.	.	.	T	C	-0.11	.	.	F	1.65	0.96
Gly	357	.	.	.	.	.	T	C	0.39	.	.	F	1.95	0.96
Ala	358	.	.	.	.	.	.	C	1.24	.	.	F	2.20	1.37
Asp	359	.	.	.	.	.	T	C	1.14	.	.	F	3.00	1.48
Pro	360	A	.	.	.	.	T	.	0.92	*	.	F	2.50	2.16
Thr	361	A	.	.	.	.	T	.	0.32	.	.	F	1.90	1.76
Glu	362	A	.	.	.	.	T	.	-0.14	.	.	F	1.60	1.04
Thr	363	A	.	.	B	.	.	.	-0.26	.	.	F	0.15	0.56
Leu	364	A	.	.	B	.	.	.	-0.96	*	.	.	-0.60	0.33
Met	365	A	.	.	B	.	.	.	-0.74	*	.	.	-0.60	0.17
Leu	366	A	.	.	B	.	.	.	-0.39	*	.	.	-0.60	0.19
Phe	367	A	.	.	B	.	.	.	-1.09	*	.	.	-0.60	0.47
Phe	368	A	.	.	B	.	.	.	-1.37	*	.	.	-0.60	0.41
Asp	369	A	.	.	B	.	.	.	-0.56	*	.	.	-0.60	0.50
Lys	370	A	A	.	.	.	.	.	-0.84	*	.	.	-0.30	0.93
Phe	371	A	A	.	B	.	.	.	-0.89	*	.	.	-0.30	0.75
Ala	372	A	A	.	B	.	.	.	-0.40	*	.	.	-0.30	0.34
Asn	373	.	A	B	B	.	.	.	-0.40	*	.	.	-0.60	0.26
Ile	374	.	A	B	B	.	.	.	-0.40	*	.	.	-0.60	0.26
Val	375	.	A	B	B	.	.	.	-0.74	.	.	.	-0.60	0.43
Pro	376	.	A	.	B	.	.	C	-0.33	.	.	.	-0.10	0.36
Phe	377	.	.	.	.	T	T	.	0.26	.	.	.	0.20	0.54
Asp	378	.	.	.	.	T	T	.	0.26	.	.	F	0.80	1.21
Ser	379	.	.	.	.	T	T	.	0.33	.	.	F	1.40	1.35
Trp	380	A	.	.	.	.	T	.	0.59	*	*	F	0.40	1.29
Asp	381	A	A	.	.	.	.	.	0.91	*	.	F	-0.15	0.76
Gln	382	A	A	.	.	.	.	.	1.61	*	.	.	-0.15	1.11
Leu	383	A	A	.	.	.	.	.	0.80	*	.	.	-0.15	1.84
Met	384	A	A	.	.	.	.	.	1.10	*	.	.	0.30	0.91
Arg	385	A	A	.	.	.	.	.	0.58	*	.	.	0.30	0.87
Gln	386	A	A	.	.	.	.	.	0.27	*	.	.	-0.30	0.87
Leu	387	A	A	.	.	.	.	.	0.31	*	.	.	0.45	1.27
Asp	388	A	A	.	.	.	.	.	1.12	*	.	.	0.75	1.30
Leu	389	A	A	.	.	.	.	.	1.72	*	.	F	0.60	1.21
Thr	390	A	.	.	.	.	T	.	0.72	*	.	F	1.30	2.54
Lys	391	A	.	.	.	.	T	.	0.72	.	*	F	1.30	1.07
Asn	392	A	.	.	.	.	T	.	0.68	*	*	F	1.30	2.16
Glu	393	A	.	.	.	.	T	.	-0.18	*	.	F	1.30	1.11
Ile	394	.	.	B	B	.	.	.	0.74	*	.	F	0.75	0.41
Asp	395	.	.	B	B	.	.	.	0.47	*	*	.	0.60	0.50
Val	396	.	.	B	B	.	.	.	0.08	*	*	.	0.60	0.29
Val	397	.	.	B	B	.	.	.	-0.23	.	.	.	0.51	0.41
Arg	398	.	.	B	.	.	T	.	-0.82	*	.	.	1.12	0.36
Ala	399	.	.	B	.	.	T	.	-0.28	*	.	.	0.73	0.49
Gly	400	.	.	.	.	T	T	.	-0.49	*	.	F	2.09	0.65

Table 2 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Thr	401	.	.	.	.	.	T	C	0.02	*	*	F	2.10	0.51
Ala	402	.	.	.	.	.	.	C	0.88	*	*	F	1.09	0.50
Gly	403	.	.	.	.	.	T	C	0.18	*	*	F	1.68	0.85
Pro	404	.	.	.	.	.	T	C	-0.04	.	.	F	1.47	0.59
Gly	405	.	.	.	.	.	T	C	0.06	.	.	F	1.26	0.48
Asp	406	A	.	.	.	.	T	.	-0.22	.	.	F	0.25	0.76
Ala	407	A	A	.	.	.	.	.	-0.23	.	.	.	-0.30	0.50
Leu	408	A	A	.	.	.	.	.	-0.70	.	.	.	-0.60	0.50
Tyr	409	A	A	.	.	.	.	.	-1.09	*	.	.	-0.60	0.25
Ala	410	A	A	.	.	.	.	.	-0.70	*	.	.	-0.60	0.24
Met	411	A	A	.	.	.	.	.	-0.99	*	.	.	-0.60	0.59
Leu	412	A	A	.	.	.	.	.	-1.26	*	.	.	-0.60	0.39
Met	413	A	A	.	.	.	.	.	-0.44	*	.	.	-0.60	0.29
Lys	414	A	A	.	B	.	.	.	-0.16	*	.	.	-0.60	0.47
Trp	415	A	A	.	B	.	.	.	0.12	*	.	.	0.15	1.14
Val	416	A	A	.	B	.	.	.	0.38	*	*	.	0.45	1.66
Asn	417	A	.	.	.	.	T	.	1.30	*	.	F	1.75	0.82
Lys	418	A	.	.	.	.	T	.	1.90	*	.	F	2.20	1.53
Thr	419	.	.	.	.	.	T	C	1.27	*	.	F	3.00	3.32
Gly	420	.	.	.	.	.	T	C	1.26	*	.	F	2.70	2.08
Arg	421	.	.	.	.	T	.	.	1.22	*	.	F	2.40	1.40
Asn	422	.	.	.	.	.	T	C	1.19	*	.	F	1.65	0.68
Ala	423	.	.	B	.	.	T	.	0.83	.	.	.	1.00	0.93
Ser	424	.	.	B	.	.	T	.	0.33	.	.	.	0.70	0.69
Ile	425	.	.	B	.	.	T	.	-0.13	.	*	.	-0.20	0.35
His	426	.	A	B	.	.	.	.	-0.24	.	*	.	-0.60	0.29
Thr	427	.	A	B	.	.	.	.	-0.83	*	*	.	-0.60	0.36
Leu	428	A	A	.	.	.	.	.	-1.06	*	*	.	-0.60	0.52
Leu	429	A	A	.	.	.	.	.	-0.76	*	*	.	-0.60	0.31
Asp	430	A	A	.	.	.	.	.	0.24	*	*	.	-0.30	0.38
Ala	431	A	A	.	.	.	.	.	-0.32	*	*	.	0.30	0.89
Leu	432	A	A	.	.	.	.	.	-0.01	*	*	.	0.75	1.07
Glu	433	A	A	.	.	.	.	.	0.80	*	*	.	0.75	1.11
Arg	434	A	A	.	.	.	.	.	1.72	*	*	F	0.90	1.90
Met	435	A	A	.	.	.	.	.	1.69	*	*	F	0.90	4.52
Glu	436	A	A	.	.	.	.	.	1.69	*	*	F	0.90	3.55
Glu	437	A	A	.	.	.	.	.	2.54	*	.	F	0.90	1.83
Arg	438	A	A	.	.	.	.	.	2.54	*	*	F	0.90	3.70
His	439	A	A	.	.	.	.	.	2.48	*	*	F	0.90	3.70
Ala	440	A	A	.	.	.	.	.	2.19	*	*	F	0.90	4.28
Lys	441	A	A	.	.	.	.	.	2.19	*	*	F	0.90	1.53
Glu	442	A	A	.	.	.	.	.	2.19	*	.	F	0.90	1.95
Lys	443	A	A	.	.	.	.	.	1.27	*	*	F	0.90	3.22
Ile	444	A	A	.	.	.	.	.	0.49	*	*	F	0.90	1.33
Gln	445	A	A	.	.	.	.	.	0.22	*	*	F	0.75	0.63
Asp	446	A	A	.	.	.	.	.	0.18	*	*	F	-0.15	0.23
Leu	447	A	A	.	.	.	.	.	-0.12	*	.	.	-0.30	0.56
Leu	448	A	A	.	.	.	.	.	-0.51	*	.	.	0.55	0.43
Val	449	A	A	.	.	.	.	.	0.42	*	.	F	0.95	0.26
Asp	450	A	.	.	.	.	T	.	-0.28	*	.	F	1.60	0.62

**Table 2 (continued)**

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ser	451	.	.	.	.	T	T	.	-1.17	*	.	F	2.25	0.65
Gly	452	.	.	.	.	T	T	.	-0.60	*	.	F	2.50	0.62
Lys	453	.	.	B	.	.	T	.	-0.60	.	.	F	1.25	0.58
Phe	454	.	A	B	.	.	.	.	0.26	.	.	.	0.15	0.36
Ile	455	.	A	B	.	.	.	.	0.26	.	.	.	0.20	0.62
Tyr	456	.	A	B	.	.	.	.	0.21	.	.	.	0.55	0.52
Leu	457	.	A	B	.	.	.	.	0.24	.	.	.	-0.03	0.59
Glu	458	.	A	B	.	.	.	.	-0.14	.	.	F	0.54	1.22
Asp	459	.	A	.	.	T	.	.	0.26	.	.	F	1.66	0.77
Gly	460	.	.	.	.	T	T	.	0.56	.	.	F	2.78	1.26
Thr	461	.	.	.	.	.	T	C	-0.06	*	.	F	2.70	0.73
Gly	462	.	.	.	.	.	T	C	0.46	*	.	F	2.13	0.33
Ser	463	.	.	.	.	.	T	C	-0.36	.	.	F	1.26	0.44
Ala	464	A	.	.	.	.	.	.	-0.36	.	.	.	0.14	0.25
Val	465	.	.	B	.	.	.	.	-0.40	.	.	.	0.17	0.44
Ser	466	.	.	B	.	.	.	.	-0.48	.	.	.	-0.10	0.42
Leu	467	.	.	B	.	.	.	.	-0.52	.	.	.	-0.10	0.53
Glu	468	A	.	.	.	.	.	.	-0.61	.	.	.	0.50	0.92

[0085] In another aspect, the invention provides an antibody that binds a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide described herein. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).

[0086] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science* 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0087] Antigenic epitope-bearing peptides and polypeptides are therefore useful to raise antibodies, including monoclonal antibodies, that bind to a TR4 polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of SEQ ID NO:1.

[0088] Antibodies of the invention may bind one or more antigenic TR4 polypeptides or peptides including, but not limited to: a polypeptide comprising amino acid residues from about 35 to about 92 of SEQ ID NO:1; a polypeptide comprising amino acid residues from about 114 to about 160 of SEQ ID NO:1; a polypeptide comprising amino acid residues from about 169 to about 240 of SEQ ID NO:1; a polypeptide comprising amino acid residues from about 267 to about 298 of SEQ ID NO:1; a polypeptide comprising amino acid residues from about 330 to about 364 of SEQ ID NO:1; a polypeptide comprising amino acid residues from about 391 to about 404 of SEQ ID NO:1; and/or a

polypeptide comprising amino acid residues from about 418 to about 465 of SEQ ID NO:1. In this context "about" includes the particularly recited range, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the TR4 protein. Epitope-bearing TR4 peptides and polypeptides may be produced by any conventional means. Houghten, R.A., "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids," *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

[0089] As one of skill in the art will appreciate, TR4 polypeptides and the epitope-bearing fragments thereof described herein (e.g., corresponding to a portion of the extracellular domain such as, for example, amino acid residues 1 to 240 of SEQ ID NO:1 can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*, *Nature* 331:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric TR4 protein or protein fragment alone (Fountoulakis *et al.*, *J Biochem* 270:3958-3964 (1995)). Thus, antibodies of the invention may bind fusion proteins that comprise all or a portion of a TR4 polypeptide such as TR4.

[0090] Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may also bind such modified TR4 polypeptides or TR4 polypeptide fragments or variants.

[0091] For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus

without substantial loss of biological function, or loss of the ability to be bound by a specific antibody. For instance, Ron *et al.*, *J. Biol. Chem.*, 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. In the present case, since TR4 is a member of the death domain containing receptor (DDCR) polypeptide family, deletions of N-terminal amino acids up to the cysteine residue at position 109 in SEQ ID NO:1 may retain some biological activity such as the ability to induce apoptosis. Polypeptides having further N-terminal deletions including the cysteine residue at position 109 (C-109) in SEQ ID NO:1 would not be expected to retain such biological activities because this residue is conserved among family members and may be required for forming a disulfide bridge to provide structural stability which is needed for ligand binding.

[0092] However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind TR4 ligand (e.g., TRAIL)) may still be retained. For example, the ability of shortened TR4 polypeptides to induce and/or bind to antibodies which recognize the complete or mature forms of the TR4 polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a TR4 polypeptide with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six TR4 amino acid residues may often evoke an immune response.

[0093] Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the amino terminus of the TR4 amino acid sequence of SEQ ID NO:1 up to the serine residue at position number 463 and polynucleotides encoding such polypeptides. In particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues  $n^1$ -468 of SEQ ID NO:1, where  $n^1$  is an integer from 2 to 463 corresponding to the position of the amino acid residue in SEQ ID NO:1.

[0094] More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of A-2 to

E-468; P-3 to E-468; P-4 to E-468; P-5 to E-468; A-6 to E-468; R-7 to E-468; V-8 to E-468; H-9 to E-468; L-10 to E-468; G-11 to E-468; A-12 to E-468; F-13 to E-468; L-14 to E-468; A-15 to E-468; V-16 to E-468; T-17 to E-468; P-18 to E-468; N-19 to E-468; P-20 to E-468; G-21 to E-468; S-22 to E-468; A-23 to E-468; A-24 to E-468; S-25 to E-468; G-26 to E-468; T-27 to E-468; E-28 to E-468; A-29 to E-468; A-30 to E-468; A-31 to E-468; A-32 to E-468; T-33 to E-468; P-34 to E-468; S-35 to E-468; K-36 to E-468; V-37 to E-468; W-38 to E-468; G-39 to E-468; S-40 to E-468; S-41 to E-468; A-42 to E-468; G-43 to E-468; R-44 to E-468; I-45 to E-468; E-46 to E-468; P-47 to E-468; R-48 to E-468; G-49 to E-468; G-50 to E-468; G-51 to E-468; R-52 to E-468; G-53 to E-468; A-54 to E-468; L-55 to E-468; P-56 to E-468; T-57 to E-468; S-58 to E-468; M-59 to E-468; G-60 to E-468; Q-61 to E-468; H-62 to E-468; G-63 to E-468; P-64 to E-468; S-65 to E-468; A-66 to E-468; R-67 to E-468; A-68 to E-468; R-69 to E-468; A-70 to E-468; G-71 to E-468; R-72 to E-468; A-73 to E-468; P-74 to E-468; G-75 to E-468; P-76 to E-468; R-77 to E-468; P-78 to E-468; A-79 to E-468; R-80 to E-468; E-81 to E-468; A-82 to E-468; S-83 to E-468; P-84 to E-468; R-85 to E-468; L-86 to E-468; R-87 to E-468; V-88 to E-468; H-89 to E-468; K-90 to E-468; T-91 to E-468; F-92 to E-468; K-93 to E-468; F-94 to E-468; V-95 to E-468; V-96 to E-468; V-97 to E-468; G-98 to E-468; V-99 to E-468; L-100 to E-468; L-101 to E-468; Q-102 to E-468; V-103 to E-468; V-104 to E-468; P-105 to E-468; S-106 to E-468; S-107 to E-468; A-108 to E-468; A-109 to E-468; T-110 to E-468; I-111 to E-468; K-112 to E-468; L-113 to E-468; H-114 to E-468; D-115 to E-468; Q-116 to E-468; S-117 to E-468; I-118 to E-468; G-119 to E-468; T-120 to E-468; Q-121 to E-468; Q-122 to E-468; W-123 to E-468; E-124 to E-468; H-125 to E-468; S-126 to E-468; P-127 to E-468; L-128 to E-468; G-129 to E-468; E-130 to E-468; L-131 to E-468; C-132 to E-468; P-133 to E-468; P-134 to E-468; G-135 to E-468; S-136 to E-468; H-137 to E-468; R-138 to E-468; S-139 to E-468; E-140 to E-468; R-141 to E-468; P-142 to E-468; G-143 to E-468; A-144 to E-468; C-145 to E-468; N-146 to E-468; R-147 to E-468; C-148 to E-468; T-149 to E-468; E-150 to E-468; G-151 to E-468; V-152 to E-468; G-153 to E-468; Y-154 to E-468; T-155 to E-468; N-156 to E-468; A-157 to E-468; S-158 to E-468; N-159 to E-468; N-160 to E-468; L-161 to E-468; F-162 to E-468; A-163 to E-468; C-164 to E-468; L-165 to E-468; P-166 to E-468; C-167 to E-468; T-168 to E-468; A-169 to E-468; C-170 to E-468; K-171 to E-468; S-172 to E-468; D-173 to E-468; E-174 to E-468; E-175 to E-468; E-176 to E-468; R-177 to E-468; S-178 to E-468; P-179 to E-468; C-180 to E-468; T-181 to E-468; T-182 to E-468; T-183 to E-468; R-184 to E-468; N-185 to E-468;

T-186 to E-468; A-187 to E-468; C-188 to E-468; Q-189 to E-468; C-190 to E-468; K-191 to E-468; P-192 to E-468; G-193 to E-468; T-194 to E-468; F-195 to E-468; R-196 to E-468; N-197 to E-468; D-198 to E-468; N-199 to E-468; S-200 to E-468; A-201 to E-468; E-202 to E-468; M-203 to E-468; C-204 to E-468; R-205 to E-468; K-206 to E-468; C-207 to E-468; S-208 to E-468; T-209 to E-468; G-210 to E-468; C-211 to E-468; P-212 to E-468; R-213 to E-468; G-214 to E-468; M-215 to E-468; V-216 to E-468; K-217 to E-468; V-218 to E-468; K-219 to E-468; D-220 to E-468; C-221 to E-468; T-222 to E-468; P-223 to E-468; W-224 to E-468; S-225 to E-468; D-226 to E-468; I-227 to E-468; E-228 to E-468; C-229 to E-468; V-230 to E-468; H-231 to E-468; K-232 to E-468; E-233 to E-468; S-234 to E-468; G-235 to E-468; N-236 to E-468; G-237 to E-468; H-238 to E-468; N-239 to E-468; I-240 to E-468; W-241 to E-468; V-242 to E-468; I-243 to E-468; L-244 to E-468; V-245 to E-468; V-246 to E-468; T-247 to E-468; L-248 to E-468; V-249 to E-468; V-250 to E-468; P-251 to E-468; L-252 to E-468; L-253 to E-468; L-254 to E-468; V-255 to E-468; A-256 to E-468; V-257 to E-468; L-258 to E-468; I-259 to E-468; V-260 to E-468; C-261 to E-468; C-262 to E-468; C-263 to E-468; I-264 to E-468; G-265 to E-468; S-266 to E-468; G-267 to E-468; C-268 to E-468; G-269 to E-468; G-270 to E-468; D-271 to E-468; P-272 to E-468; K-273 to E-468; C-274 to E-468; M-275 to E-468; D-276 to E-468; R-277 to E-468; V-278 to E-468; C-279 to E-468; F-280 to E-468; W-281 to E-468; R-282 to E-468; L-283 to E-468; G-284 to E-468; L-285 to E-468; L-286 to E-468; R-287 to E-468; G-288 to E-468; P-289 to E-468; G-290 to E-468; A-291 to E-468; E-292 to E-468; D-293 to E-468; N-294 to E-468; A-295 to E-468; H-296 to E-468; N-297 to E-468; E-298 to E-468; I-299 to E-468; L-300 to E-468; S-301 to E-468; N-302 to E-468; A-303 to E-468; D-304 to E-468; S-305 to E-468; L-306 to E-468; S-307 to E-468; T-308 to E-468; F-309 to E-468; V-310 to E-468; S-311 to E-468; E-312 to E-468; Q-313 to E-468; Q-314 to E-468; M-315 to E-468; E-316 to E-468; S-317 to E-468; Q-318 to E-468; E-319 to E-468; P-320 to E-468; A-321 to E-468; D-322 to E-468; L-323 to E-468; T-324 to E-468; G-325 to E-468; V-326 to E-468; T-327 to E-468; V-328 to E-468; Q-329 to E-468; S-330 to E-468; P-331 to E-468; G-332 to E-468; E-333 to E-468; A-334 to E-468; Q-335 to E-468; C-336 to E-468; L-337 to E-468; L-338 to E-468; G-339 to E-468; P-340 to E-468; A-341 to E-468; E-342 to E-468; A-343 to E-468; E-344 to E-468; G-345 to E-468; S-346 to E-468; Q-347 to E-468; R-348 to E-468; R-349 to E-468; R-350 to E-468; L-351 to E-468; L-352 to E-468; V-353 to E-468; P-354 to E-468; A-355 to E-468; N-356 to E-468; G-357 to E-468; A-358 to E-468; D-359 to E-468; P-360 to E-468; T-361



to E-468; E-362 to E-468; T-363 to E-468; L-364 to E-468; M-365 to E-468; L-366 to E-468; F-367 to E-468; F-368 to E-468; D-369 to E-468; K-370 to E-468; F-371 to E-468; A-372 to E-468; N-373 to E-468; I-374 to E-468; V-375 to E-468; P-376 to E-468; F-377 to E-468; D-378 to E-468; S-379 to E-468; W-380 to E-468; D-381 to E-468; Q-382 to E-468; L-383 to E-468; M-384 to E-468; R-385 to E-468; Q-386 to E-468; L-387 to E-468; D-388 to E-468; L-389 to E-468; T-390 to E-468; K-391 to E-468; N-392 to E-468; E-393 to E-468; I-394 to E-468; D-395 to E-468; V-396 to E-468; V-397 to E-468; R-398 to E-468; A-399 to E-468; G-400 to E-468; T-401 to E-468; A-402 to E-468; G-403 to E-468; P-404 to E-468; G-405 to E-468; D-406 to E-468; A-407 to E-468; L-408 to E-468; Y-409 to E-468; A-410 to E-468; M-411 to E-468; L-412 to E-468; M-413 to E-468; K-414 to E-468; W-415 to E-468; V-416 to E-468; N-417 to E-468; K-418 to E-468; T-419 to E-468; G-420 to E-468; R-421 to E-468; N-422 to E-468; A-423 to E-468; S-424 to E-468; I-425 to E-468; H-426 to E-468; T-427 to E-468; L-428 to E-468; L-429 to E-468; D-430 to E-468; A-431 to E-468; L-432 to E-468; E-433 to E-468; R-434 to E-468; M-435 to E-468; E-436 to E-468; E-437 to E-468; R-438 to E-468; H-439 to E-468; A-440 to E-468; K-441 to E-468; E-442 to E-468; K-443 to E-468; I-444 to E-468; Q-445 to E-468; D-446 to E-468; L-447 to E-468; L-448 to E-468; V-449 to E-468; D-450 to E-468; S-451 to E-468; G-452 to E-468; K-453 to E-468; F-454 to E-468; I-455 to E-468; Y-456 to E-468; L-457 to E-468; E-458 to E-468; D-459 to E-468; G-460 to E-468; T-461 to E-468; G-462 to E-468; and/or S-463 to E-468 of the TR4 sequence of SEQ ID NO:1.

**[0095]** In another embodiment, N-terminal deletions of the TR4 polypeptide can be described by the general formula  $n^2$  to 238 where  $n^2$  is a number from 2 to 238 corresponding to the amino acid sequence identified of SEQ ID NO:1. In specific embodiments, antibodies of the invention bind N terminal deletions of the TR4 comprising, or alternatively consisting of, the amino acid sequence of residues: A-2 to H-238; P-3 to H-238; P-4 to H-238; P-5 to H-238; A-6 to H-238; R-7 to H-238; V-8 to H-238; H-9 to H-238; L-10 to H-238; G-11 to H-238; A-12 to H-238; F-13 to H-238; L-14 to H-238; A-15 to H-238; V-16 to H-238; T-17 to H-238; P-18 to H-238; N-19 to H-238; P-20 to H-238; G-21 to H-238; S-22 to H-238; A-23 to H-238; A-24 to H-238; S-25 to H-238; G-26 to H-238; T-27 to H-238; E-28 to H-238; A-29 to H-238; A-30 to H-238; A-31 to H-238; A-32 to H-238; T-33 to H-238; P-34 to H-238; S-35 to H-238; K-36 to H-238; V-37 to H-238; W-38 to H-238; G-39 to H-238; S-40 to H-238; S-41 to H-238; A-42 to H-238; G-43 to H-238; R-44 to H-238; I-45 to H-238; E-46 to H-238; P-47 to H-238; R-48

to H-238; G-49 to H-238; G-50 to H-238; G-51 to H-238; R-52 to H-238; G-53 to H-238; A-54 to H-238; L-55 to H-238; P-56 to H-238; T-57 to H-238; S-58 to H-238; M-59 to H-238; G-60 to H-238; Q-61 to H-238; H-62 to H-238; G-63 to H-238; P-64 to H-238; S-65 to H-238; A-66 to H-238; R-67 to H-238; A-68 to H-238; R-69 to H-238; A-70 to H-238; G-71 to H-238; R-72 to H-238; A-73 to H-238; P-74 to H-238; G-75 to H-238; P-76 to H-238; R-77 to H-238; P-78 to H-238; A-79 to H-238; R-80 to H-238; E-81 to H-238; A-82 to H-238; S-83 to H-238; P-84 to H-238; R-85 to H-238; L-86 to H-238; R-87 to H-238; V-88 to H-238; H-89 to H-238; K-90 to H-238; T-91 to H-238; F-92 to H-238; K-93 to H-238; F-94 to H-238; V-95 to H-238; V-96 to H-238; V-97 to H-238; G-98 to H-238; V-99 to H-238; L-100 to H-238; L-101 to H-238; Q-102 to H-238; V-103 to H-238; V-104 to H-238; P-105 to H-238; S-106 to H-238; S-107 to H-238; A-108 to H-238; A-109 to H-238; T-110 to H-238; I-111 to H-238; K-112 to H-238; L-113 to H-238; H-114 to H-238; D-115 to H-238; Q-116 to H-238; S-117 to H-238; I-118 to H-238; G-119 to H-238; T-120 to H-238; Q-121 to H-238; Q-122 to H-238; W-123 to H-238; E-124 to H-238; H-125 to H-238; S-126 to H-238; P-127 to H-238; L-128 to H-238; G-129 to H-238; E-130 to H-238; L-131 to H-238; C-132 to H-238; P-133 to H-238; P-134 to H-238; G-135 to H-238; S-136 to H-238; H-137 to H-238; R-138 to H-238; S-139 to H-238; E-140 to H-238; R-141 to H-238; P-142 to H-238; G-143 to H-238; A-144 to H-238; C-145 to H-238; N-146 to H-238; R-147 to H-238; C-148 to H-238; T-149 to H-238; E-150 to H-238; G-151 to H-238; V-152 to H-238; G-153 to H-238; Y-154 to H-238; T-155 to H-238; N-156 to H-238; A-157 to H-238; S-158 to H-238; N-159 to H-238; N-160 to H-238; L-161 to H-238; F-162 to H-238; A-163 to H-238; C-164 to H-238; L-165 to H-238; P-166 to H-238; C-167 to H-238; T-168 to H-238; A-169 to H-238; C-170 to H-238; K-171 to H-238; S-172 to H-238; D-173 to H-238; E-174 to H-238; E-175 to H-238; E-176 to H-238; R-177 to H-238; S-178 to H-238; P-179 to H-238; C-180 to H-238; T-181 to H-238; T-182 to H-238; T-183 to H-238; R-184 to H-238; N-185 to H-238; T-186 to H-238; A-187 to H-238; C-188 to H-238; Q-189 to H-238; C-190 to H-238; K-191 to H-238; P-192 to H-238; G-193 to H-238; T-194 to H-238; F-195 to H-238; R-196 to H-238; N-197 to H-238; D-198 to H-238; N-199 to H-238; S-200 to H-238; A-201 to H-238; E-202 to H-238; M-203 to H-238; C-204 to H-238; R-205 to H-238; K-206 to H-238; C-207 to H-238; S-208 to H-238; T-209 to H-238; G-210 to H-238; C-211 to H-238; P-212 to H-238; R-213 to H-238; G-214 to H-238; M-215 to H-238; V-216 to H-238; K-217 to H-238; V-218 to H-238; K-219 to H-238; D-220 to H-238; C-221 to H-238; T-222 to H-238; P-223 to H-238; W-224 to H-

238; S-225 to H-238; D-226 to H-238; I-227 to H-238; E-228 to H-238; C-229 to H-238; V-230 to H-238; H-231 to H-238; K-232 to H-238; and/or E-233 to H-238; of the TR4 extracellular domain sequence of SEQ ID NO:1.

**[0096]** As mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind DR4 ligand (e.g., TRAIL)) may still be retained. For example the ability of the shortened TR4 polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the TR4 polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a TR4 polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six TR4 amino acid residues may often evoke an immune response.

**[0097]** Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the TR4 polypeptide sequence of SEQ ID NO:1 up to the alanine residue at position number 30, and polynucleotides encoding such polypeptides. In particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues 24-m<sup>1</sup> of SEQ ID NO:1, where m<sup>1</sup> is an integer from 30 to 467 corresponding to the position of the amino acid residue in SEQ ID NO:1.

**[0098]** More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues A-24 to L-467; A-24 to S-466; A-24 to V-465; A-24 to A-464; A-24 to S-463; A-24 to G-462; A-24 to T-461; A-24 to G-460; A-24 to D-459; A-24 to E-458; A-24 to L-457; A-24 to Y-456; A-24 to I-455; A-24 to F-454; A-24 to K-453; A-24 to G-452; A-24 to S-451; A-24 to D-450; A-24 to V-449; A-24 to L-448; A-24 to L-447; A-24 to D-446; A-24 to Q-445; A-24 to I-444; A-24 to K-443; A-24 to E-442; A-24 to K-441; A-24 to A-440; A-24 to H-439; A-24 to R-438; A-24 to E-437; A-24 to E-436; A-24 to M-435; A-24 to R-434; A-24 to E-433; A-24 to L-432; A-24 to A-431; A-24 to D-430; A-24 to L-429; A-24 to L-428; A-24 to T-427; A-24 to H-426; A-24 to I-425; A-24 to S-424; A-24 to A-423; A-24 to N-422;

A-24 to R-421; A-24 to G-420; A-24 to T-419; A-24 to K-418; A-24 to N-417; A-24 to V-416; A-24 to W-415; A-24 to K-414; A-24 to M-413; A-24 to L-412; A-24 to M-411; A-24 to A-410; A-24 to Y-409; A-24 to L-408; A-24 to A-407; A-24 to D-406; A-24 to G-405; A-24 to P-404; A-24 to G-403; A-24 to A-402; A-24 to T-401; A-24 to G-400; A-24 to A-399; A-24 to R-398; A-24 to V-397; A-24 to V-396; A-24 to D-395; A-24 to I-394; A-24 to E-393; A-24 to N-392; A-24 to K-391; A-24 to T-390; A-24 to L-389; A-24 to D-388; A-24 to L-387; A-24 to Q-386; A-24 to R-385; A-24 to M-384; A-24 to L-383; A-24 to Q-382; A-24 to D-381; A-24 to W-380; A-24 to S-379; A-24 to D-378; A-24 to F-377; A-24 to P-376; A-24 to V-375; A-24 to I-374; A-24 to N-373; A-24 to A-372; A-24 to F-371; A-24 to K-370; A-24 to D-369; A-24 to F-368; A-24 to F-367; A-24 to L-366; A-24 to M-365; A-24 to L-364; A-24 to T-363; A-24 to E-362; A-24 to T-361; A-24 to P-360; A-24 to D-359; A-24 to A-358; A-24 to G-357; A-24 to N-356; A-24 to A-355; A-24 to P-354; A-24 to V-353; A-24 to L-352; A-24 to L-351; A-24 to R-350; A-24 to R-349; A-24 to R-348; A-24 to Q-347; A-24 to S-346; A-24 to G-345; A-24 to E-344; A-24 to A-343; A-24 to E-342; A-24 to A-341; A-24 to P-340; A-24 to G-339; A-24 to L-338; A-24 to L-337; A-24 to C-336; A-24 to Q-335; A-24 to A-334; A-24 to E-333; A-24 to G-332; A-24 to P-331; A-24 to S-330; A-24 to Q-329; A-24 to V-328; A-24 to T-327; A-24 to V-326; A-24 to G-325; A-24 to T-324; A-24 to L-323; A-24 to D-322; A-24 to A-321; A-24 to P-320; A-24 to E-319; A-24 to Q-318; A-24 to S-317; A-24 to E-316; A-24 to M-315; A-24 to Q-314; A-24 to Q-313; A-24 to E-312; A-24 to S-311; A-24 to V-310; A-24 to F-309; A-24 to T-308; A-24 to S-307; A-24 to L-306; A-24 to S-305; A-24 to D-304; A-24 to A-303; A-24 to N-302; A-24 to S-301; A-24 to L-300; A-24 to I-299; A-24 to E-298; A-24 to N-297; A-24 to H-296; A-24 to A-295; A-24 to N-294; A-24 to D-293; A-24 to E-292; A-24 to A-291; A-24 to G-290; A-24 to P-289; A-24 to G-288; A-24 to R-287; A-24 to L-286; A-24 to L-285; A-24 to G-284; A-24 to L-283; A-24 to R-282; A-24 to W-281; A-24 to F-280; A-24 to C-279; A-24 to V-278; A-24 to R-277; A-24 to D-276; A-24 to M-275; A-24 to C-274; A-24 to K-273; A-24 to P-272; A-24 to D-271; A-24 to G-270; A-24 to G-269; A-24 to C-268; A-24 to G-267; A-24 to S-266; A-24 to G-265; A-24 to I-264; A-24 to C-263; A-24 to C-262; A-24 to C-261; A-24 to V-260; A-24 to I-259; A-24 to L-258; A-24 to V-257; A-24 to A-256; A-24 to V-255; A-24 to L-254; A-24 to L-253; A-24 to L-252; A-24 to P-251; A-24 to V-250; A-24 to V-249; A-24 to L-248; A-24 to T-247; A-24 to V-246; A-24 to V-245; A-24 to L-244; A-24 to I-243; A-24 to V-242; A-24 to W-241; A-24 to I-240; A-24 to N-239; A-24 to H-238; A-24 to G-237; A-24 to N-236; A-24 to G-

235; A-24 to S-234; A-24 to E-233; A-24 to K-232; A-24 to H-231; A-24 to V-230; A-24 to C-229; A-24 to E-228; A-24 to I-227; A-24 to D-226; A-24 to S-225; A-24 to W-224; A-24 to P-223; A-24 to T-222; A-24 to C-221; A-24 to D-220; A-24 to K-219; A-24 to V-218; A-24 to K-217; A-24 to V-216; A-24 to M-215; A-24 to G-214; A-24 to R-213; A-24 to P-212; A-24 to C-211; A-24 to G-210; A-24 to T-209; A-24 to S-208; A-24 to C-207; A-24 to K-206; A-24 to R-205; A-24 to C-204; A-24 to M-203; A-24 to E-202; A-24 to A-201; A-24 to S-200; A-24 to N-199; A-24 to D-198; A-24 to N-197; A-24 to R-196; A-24 to F-195; A-24 to T-194; A-24 to G-193; A-24 to P-192; A-24 to K-191; A-24 to C-190; A-24 to Q-189; A-24 to C-188; A-24 to A-187; A-24 to T-186; A-24 to N-185; A-24 to R-184; A-24 to T-183; A-24 to T-182; A-24 to T-181; A-24 to C-180; A-24 to P-179; A-24 to S-178; A-24 to R-177; A-24 to E-176; A-24 to E-175; A-24 to E-174; A-24 to D-173; A-24 to S-172; A-24 to K-171; A-24 to C-170; A-24 to A-169; A-24 to T-168; A-24 to C-167; A-24 to P-166; A-24 to L-165; A-24 to C-164; A-24 to A-163; A-24 to F-162; A-24 to L-161; A-24 to N-160; A-24 to N-159; A-24 to S-158; A-24 to A-157; A-24 to N-156; A-24 to T-155; A-24 to Y-154; A-24 to G-153; A-24 to V-152; A-24 to G-151; A-24 to E-150; A-24 to T-149; A-24 to C-148; A-24 to R-147; A-24 to N-146; A-24 to C-145; A-24 to A-144; A-24 to G-143; A-24 to P-142; A-24 to R-141; A-24 to E-140; A-24 to S-139; A-24 to R-138; A-24 to H-137; A-24 to S-136; A-24 to G-135; A-24 to P-134; A-24 to P-133; A-24 to C-132; A-24 to L-131; A-24 to E-130; A-24 to G-129; A-24 to L-128; A-24 to P-127; A-24 to S-126; A-24 to H-125; A-24 to E-124; A-24 to W-123; A-24 to Q-122; A-24 to Q-121; A-24 to T-120; A-24 to G-119; A-24 to I-118; A-24 to S-117; A-24 to Q-116; A-24 to D-115; A-24 to H-114; A-24 to L-113; A-24 to K-112; A-24 to I-111; A-24 to T-110; A-24 to A-109; A-24 to A-108; A-24 to S-107; A-24 to S-106; A-24 to P-105; A-24 to V-104; A-24 to V-103; A-24 to Q-102; A-24 to L-101; A-24 to L-100; A-24 to V-99; A-24 to G-98; A-24 to V-97; A-24 to V-96; A-24 to V-95; A-24 to F-94; A-24 to K-93; A-24 to F-92; A-24 to T-91; A-24 to K-90; A-24 to H-89; A-24 to V-88; A-24 to R-87; A-24 to L-86; A-24 to R-85; A-24 to P-84; A-24 to S-83; A-24 to A-82; A-24 to E-81; A-24 to R-80; A-24 to A-79; A-24 to P-78; A-24 to R-77; A-24 to P-76; A-24 to G-75; A-24 to P-74; A-24 to A-73; A-24 to R-72; A-24 to G-71; A-24 to A-70; A-24 to R-69; A-24 to A-68; A-24 to R-67; A-24 to A-66; A-24 to S-65; A-24 to P-64; A-24 to G-63; A-24 to H-62; A-24 to Q-61; A-24 to G-60; A-24 to M-59; A-24 to S-58; A-24 to T-57; A-24 to P-56; A-24 to L-55; A-24 to A-54; A-24 to G-53; A-24 to R-52; A-24 to G-51; A-24 to G-50; A-24 to G-49; A-24 to R-48; A-24 to P-47; A-24 to E-46; A-24 to I-45; A-24 to R-44;

A-24 to G-43; A-24 to A-42; A-24 to S-41; A-24 to S-40; A-24 to G-39; A-24 to W-38; A-24 to V-37; A-24 to K-36; A-24 to S-35; A-24 to P-34; A-24 to T-33; A-24 to A-32; A-24 to A-31; and/or A-24 to A-30 of the TR4 sequence of SEQ ID NO:1.

**[0099]** In another embodiment, antibodies of the invention bind C-terminal deletions of the TR4 polypeptide that can be described by the general formula 24-m<sup>2</sup> where m<sup>2</sup> is a number from 30 to 238 corresponding to the amino acid sequence identified of SEQ ID NO:1. In specific embodiments, the invention provides antibodies that bind TR4 polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: A-24 to G-237; A-24 to N-236; A-24 to G-235; A-24 to S-234; A-24 to E-233; A-24 to K-232; A-24 to H-231; A-24 to V-230; A-24 to C-229; A-24 to E-228; A-24 to I-227; A-24 to D-226; A-24 to S-225; A-24 to W-224; A-24 to P-223; A-24 to T-222; A-24 to C-221; A-24 to D-220; A-24 to K-219; A-24 to V-218; A-24 to K-217; A-24 to V-216; A-24 to M-215; A-24 to G-214; A-24 to R-213; A-24 to P-212; A-24 to C-211; A-24 to G-210; A-24 to T-209; A-24 to S-208; A-24 to C-207; A-24 to K-206; A-24 to R-205; A-24 to C-204; A-24 to M-203; A-24 to E-202; A-24 to A-201; A-24 to S-200; A-24 to N-199; A-24 to D-198; A-24 to N-197; A-24 to R-196; A-24 to F-195; A-24 to T-194; A-24 to G-193; A-24 to P-192; A-24 to K-191; A-24 to C-190; A-24 to Q-189; A-24 to C-188; A-24 to A-187; A-24 to T-186; A-24 to N-185; A-24 to R-184; A-24 to T-183; A-24 to T-182; A-24 to T-181; A-24 to C-180; A-24 to P-179; A-24 to S-178; A-24 to R-177; A-24 to E-176; A-24 to E-175; A-24 to E-174; A-24 to D-173; A-24 to S-172; A-24 to K-171; A-24 to C-170; A-24 to A-169; A-24 to T-168; A-24 to C-167; A-24 to P-166; A-24 to L-165; A-24 to C-164; A-24 to A-163; A-24 to F-162; A-24 to L-161; A-24 to N-160; A-24 to N-159; A-24 to S-158; A-24 to A-157; A-24 to N-156; A-24 to T-155; A-24 to Y-154; A-24 to G-153; A-24 to V-152; A-24 to G-151; A-24 to E-150; A-24 to T-149; A-24 to C-148; A-24 to R-147; A-24 to N-146; A-24 to C-145; A-24 to A-144; A-24 to G-143; A-24 to P-142; A-24 to R-141; A-24 to E-140; A-24 to S-139; A-24 to R-138; A-24 to H-137; A-24 to S-136; A-24 to G-135; A-24 to P-134; A-24 to P-133; A-24 to C-132; A-24 to L-131; A-24 to E-130; A-24 to G-129; A-24 to L-128; A-24 to P-127; A-24 to S-126; A-24 to H-125; A-24 to E-124; A-24 to W-123; A-24 to Q-122; A-24 to Q-121; A-24 to T-120; A-24 to G-119; A-24 to I-118; A-24 to S-117; A-24 to Q-116; A-24 to D-115; A-24 to H-114; A-24 to L-113; A-24 to K-112; A-24 to I-111; A-24 to T-110; A-24 to A-109; A-24 to A-108; A-24 to S-107; A-24 to S-106; A-24 to P-105; A-24 to V-104; A-24 to V-103; A-24 to Q-102; A-24 to L-101; A-24 to L-100; A-24 to V-99; A-24 to G-98; A-24 to V-97; A-

24 to V-96; A-24 to V-95; A-24 to F-94; A-24 to K-93; A-24 to F-92; A-24 to T-91; A-24 to K-90; A-24 to H-89; A-24 to V-88; A-24 to R-87; A-24 to L-86; A-24 to R-85; A-24 to P-84; A-24 to S-83; A-24 to A-82; A-24 to E-81; A-24 to R-80; A-24 to A-79; A-24 to P-78; A-24 to R-77; A-24 to P-76; A-24 to G-75; A-24 to P-74; A-24 to A-73; A-24 to R-72; A-24 to G-71; A-24 to A-70; A-24 to R-69; A-24 to A-68; A-24 to R-67; A-24 to A-66; A-24 to S-65; A-24 to P-64; A-24 to G-63; A-24 to H-62; A-24 to Q-61; A-24 to G-60; A-24 to M-59; A-24 to S-58; A-24 to T-57; A-24 to P-56; A-24 to L-55; A-24 to A-54; A-24 to G-53; A-24 to R-52; A-24 to G-51; A-24 to G-50; A-24 to G-49; A-24 to R-48; A-24 to P-47; A-24 to E-46; A-24 to I-45; A-24 to R-44; A-24 to G-43; A-24 to A-42; A-24 to S-41; A-24 to S-40; A-24 to G-39; A-24 to W-38; A-24 to V-37; A-24 to K-36; A-24 to S-35; A-24 to P-34; A-24 to T-33; A-24 to A-32; A-24 to A-31; and/or A-24 to A-30; of the TR4 extracellular domain sequence of SEQ ID NO:1.

**[0100]** The present invention further provides antibodies that bind polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the TR4 polypeptide of SEQ ID NO:1, up to C-221 of SEQ ID NO:1. In particular, the present invention provides antibodies that bind polypeptides having the amino acid sequence of residues 1-m<sup>9</sup> of the amino acid sequence in SEQ ID NO:1, where m<sup>9</sup> is any integer in the range of 221-468 and residue C-221 is the position of the first residue from the C-terminus of the complete TR4 polypeptide (shown in SEQ ID NO:1) believed to be required for receptor binding activity of the TR4 protein.

**[0101]** The invention also provides antibodies that bind polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a TR4 polypeptide, which may be described generally as having residues n<sup>1</sup>- m<sup>1</sup> and/or n<sup>2</sup>- m<sup>2</sup> of SEQ ID NO:1, where n<sup>1</sup>, n<sup>2</sup>, m<sup>1</sup>, and m<sup>2</sup> are integers as described above.

**[0102]** Also included are antibodies that bind a polypeptide consisting of a portion of the complete TR4 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, where this portion excludes from 1 to about 108 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, or from 1 to about 247 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853.

[0103] Preferably, antibodies of the present invention bind fragments of TR4 comprising a portion of the extracellular domain; i.e., within residues 24-238 of SEQ ID NO:1, since any portion therein is expected to be soluble.

[0104] It will be recognized in the art that some amino acid sequence of TR4 can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

[0105] Thus, the invention further includes antibodies that bind variations of the TR4 protein which show substantial TR4 protein activity or which include regions of TR4 such as the protein fragments discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitution. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U. *et al.*, *Science* 247:1306-1310 (1990).

[0106] Thus, antibodies of the present invention may bind a fragment, derivative, or analog of the polypeptide of SEQ ID NO:1, or that encoded by the cDNA in ATCC deposit 97853. Such fragments, variants or derivatives may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

[0107] Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TR4 protein.



The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al.* *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

[0108] The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al.*, *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the antibodies of the present invention may bind a TR4 receptor that contains one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

[0109] As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 3).

**TABLE 3. Conservative Amino Acid Substitutions.**

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

**[0110]** In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of SEQ ID NO:1 and/or any of the polypeptide fragments described herein (e.g., the extracellular domain or intracellular domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2.

**[0111]** In specific embodiments, the antibodies of the invention bind TR4 polypeptides or fragments or variants thereof (especially a fragment comprising or alternatively consisting of, the extracellular soluble domain of TR4), that contains any one or more of the following conservative mutations in TR4: M1 replaced with A, G, I, L, S, T, or V; A2 replaced with G, I, L, S, T, M, or V; A6 replaced with G, I, L, S, T, M, or V; R7 replaced with H, or K; V8 replaced with A, G, I, L, S, T, or M; H9 replaced with K, or R; L10 replaced with A, G, I, S, T, M, or V; G11 replaced with A, I, L, S, T, M, or V; A12 replaced with G, I, L, S, T, M, or V; F13 replaced with W, or Y; L14 replaced with A, G, I, S, T, M, or V; A15 replaced with G, I, L, S, T, M, or V; V16 replaced with A, G, I, L, S, T, or M; T17 replaced with A, G, I, L, S, M, or V; N19 replaced with Q; G21 replaced with A, I, L, S, T, M, or V; S22 replaced with A, G, I, L, T, M, or V; A23 replaced with G, I, L, S, T, M, or V; A24 replaced with G, I, L, S, T, M, or V; S25 replaced with A, G, I, L, T, M, or V; G26 replaced with A, I, L, S, T, M, or V; T27 replaced with A, G, I, L, S, M, or V; E28 replaced with D; A29 replaced with G, I, L, S, T, M, or V; A30 replaced with G, I, L, S, T, M, or V; A31 replaced with G, I, L, S, T, M, or V; A32 replaced with G, I, L, S, T, M, or V; T33 replaced with A, G, I, L, S, M, or V; S35 replaced with A, G, I, L, T, M, or V; K36 replaced with H, or R; V37 replaced with A, G, I, L, S, T, or M; W38 replaced with F, or Y; G39 replaced with A, I, L, S, T, M, or V; S40 replaced with A, G, I, L, T, M, or V; S41 replaced with A, G, I, L, T, M, or V; A42 replaced with G, I, L, S, T, M, or V; G43 replaced with A, I, L, S, T, M, or V; R44 replaced with H, or K; I45 replaced with A, G, L, S, T, M, or V; E46 replaced with D; R48 replaced with H, or K; G49 replaced with A, I, L, S, T, M, or V; G50 replaced with A, I, L, S, T, M, or V; G51 replaced with A, I, L, S, T, M, or V; R52 replaced with H, or K; G53 replaced with A, I, L, S, T, M, or V; A54 replaced with G, I, L, S, T, M, or V; L55 replaced with A, G, I, S, T, M, or V; T57 replaced with A, G, I, L, S, M, or V; S58 replaced with A, G, I, L, T, M, or V; M59 replaced with A, G, I, L, S, T, or V; G60 replaced with A, I, L, S, T, M, or V; Q61 replaced with N; H62 replaced with K, or R; G63 replaced with A, I, L, S, T, M, or

V; S65 replaced with A, G, I, L, T, M, or V; A66 replaced with G, I, L, S, T, M, or V; R67 replaced with H, or K; A68 replaced with G, I, L, S, T, M, or V; R69 replaced with H, or K; A70 replaced with G, I, L, S, T, M, or V; G71 replaced with A, I, L, S, T, M, or V; R72 replaced with H, or K; A73 replaced with G, I, L, S, T, M, or V; G75 replaced with A, I, L, S, T, M, or V; R77 replaced with H, or K; A79 replaced with G, I, L, S, T, M, or V; R80 replaced with H, or K; E81 replaced with D; A82 replaced with G, I, L, S, T, M, or V; S83 replaced with A, G, I, L, T, M, or V; R85 replaced with H, or K; L86 replaced with A, G, I, S, T, M, or V; R87 replaced with H, or K; V88 replaced with A, G, I, L, S, T, or M; H89 replaced with K, or R; K90 replaced with H, or R; T91 replaced with A, G, I, L, S, M, or V; F92 replaced with W, or Y; K93 replaced with H, or R; F94 replaced with W, or Y; V95 replaced with A, G, I, L, S, T, or M; V96 replaced with A, G, I, L, S, T, or M; V97 replaced with A, G, I, L, S, T, or M; G98 replaced with A, I, L, S, T, M, or V; V99 replaced with A, G, I, L, S, T, or M; L100 replaced with A, G, I, S, T, M, or V; L101 replaced with A, G, I, S, T, M, or V; Q102 replaced with N; V103 replaced with A, G, I, L, S, T, or M; V104 replaced with A, G, I, L, S, T, or M; S106 replaced with A, G, I, L, T, M, or V; S107 replaced with A, G, I, L, T, M, or V; A108 replaced with G, I, L, S, T, M, or V; A109 replaced with G, I, L, S, T, M, or V; T110 replaced with A, G, I, L, S, M, or V; I111 replaced with A, G, L, S, T, M, or V; K112 replaced with H, or R; L113 replaced with A, G, I, S, T, M, or V; H114 replaced with K, or R; D115 replaced with E; Q116 replaced with N; S117 replaced with A, G, I, L, T, M, or V; I118 replaced with A, G, L, S, T, M, or V; G119 replaced with A, I, L, S, T, M, or V; T120 replaced with A, G, I, L, S, M, or V; Q121 replaced with N; Q122 replaced with N; W123 replaced with F, or Y; E124 replaced with D; H125 replaced with K, or R; S126 replaced with A, G, I, L, T, M, or V; L128 replaced with A, G, I, S, T, M, or V; G129 replaced with A, I, L, S, T, M, or V; E130 replaced with D; L131 replaced with A, G, I, S, T, M, or V; G135 replaced with A, I, L, S, T, M, or V; S136 replaced with A, G, I, L, T, M, or V; H137 replaced with K, or R; R138 replaced with H, or K; S139 replaced with A, G, I, L, T, M, or V; E140 replaced with D; R141 replaced with H, or K; G143 replaced with A, I, L, S, T, M, or V; A144 replaced with G, I, L, S, T, M, or V; N146 replaced with Q; R147 replaced with H, or K; T149 replaced with A, G, I, L, S, M, or V; E150 replaced with D; G151 replaced with A, I, L, S, T, M, or V; V152 replaced with A, G, I, L, S, T, or M; G153 replaced with A, I, L, S, T, M, or V; Y154 replaced with F, or W; T155 replaced with A, G, I, L, S, M, or V; N156 replaced with Q; A157 replaced with G, I, L, S, T, M, or V; S158 replaced with A,

G, I, L, T, M, or V; N159 replaced with Q; N160 replaced with Q; L161 replaced with A, G, I, S, T, M, or V; F162 replaced with W, or Y; A163 replaced with G, I, L, S, T, M, or V; L165 replaced with A, G, I, S, T, M, or V; T168 replaced with A, G, I, L, S, M, or V; A169 replaced with G, I, L, S, T, M, or V; K171 replaced with H, or R; S172 replaced with A, G, I, L, T, M, or V; D173 replaced with E; E174 replaced with D; E175 replaced with D; E176 replaced with D; R177 replaced with H, or K; S178 replaced with A, G, I, L, T, M, or V; T181 replaced with A, G, I, L, S, M, or V; T182 replaced with A, G, I, L, S, M, or V; T183 replaced with A, G, I, L, S, M, or V; R184 replaced with H, or K; N185 replaced with Q; T186 replaced with A, G, I, L, S, M, or V; A187 replaced with G, I, L, S, T, M, or V; Q189 replaced with N; K191 replaced with H, or R; G193 replaced with A, I, L, S, T, M, or V; T194 replaced with A, G, I, L, S, M, or V; F195 replaced with W, or Y; R196 replaced with H, or K; N197 replaced with Q; D198 replaced with E; N199 replaced with Q; S200 replaced with A, G, I, L, T, M, or V; A201 replaced with G, I, L, S, T, M, or V; E202 replaced with D; M203 replaced with A, G, I, L, S, T, or V; R205 replaced with H, or K; K206 replaced with H, or R; S208 replaced with A, G, I, L, T, M, or V; T209 replaced with A, G, I, L, S, M, or V; G210 replaced with A, I, L, S, T, M, or V; R213 replaced with H, or K; G214 replaced with A, I, L, S, T, M, or V; M215 replaced with A, G, I, L, S, T, or V; V216 replaced with A, G, I, L, S, T, or M; K217 replaced with H, or R; V218 replaced with A, G, I, L, S, T, or M; K219 replaced with H, or R; D220 replaced with E; T222 replaced with A, G, I, L, S, M, or V; W224 replaced with F, or Y; S225 replaced with A, G, I, L, T, M, or V; D226 replaced with E; I227 replaced with A, G, L, S, T, M, or V; E228 replaced with D; V230 replaced with A, G, I, L, S, T, or M; H231 replaced with K, or R; K232 replaced with H, or R; E233 replaced with D; S234 replaced with A, G, I, L, T, M, or V; G235 replaced with A, I, L, S, T, M, or V; N236 replaced with Q; G237 replaced with A, I, L, S, T, M, or V; H238 replaced with K, or R; N239 replaced with Q; I240 replaced with A, G, L, S, T, M, or V; W241 replaced with F, or Y; V242 replaced with A, G, I, L, S, T, or M; I243 replaced with A, G, L, S, T, M, or V; L244 replaced with A, G, I, S, T, M, or V; V245 replaced with A, G, I, L, S, T, or M; V246 replaced with A, G, I, L, S, T, or M; T247 replaced with A, G, I, L, S, M, or V; L248 replaced with A, G, I, S, T, M, or V; V249 replaced with A, G, I, L, S, T, or M; V250 replaced with A, G, I, L, S, T, or M; L252 replaced with A, G, I, S, T, M, or V; L253 replaced with A, G, I, S, T, M, or V; L254 replaced with A, G, I, S, T, M, or V; V255 replaced with A, G, I, L, S, T, or M; A256 replaced with G, I, L, S, T, M, or V; V257

replaced with A, G, I, L, S, T, or M; L258 replaced with A, G, I, S, T, M, or V; I259 replaced with A, G, L, S, T, M, or V; V260 replaced with A, G, I, L, S, T, or M; I264 replaced with A, G, L, S, T, M, or V; G265 replaced with A, I, L, S, T, M, or V; S266 replaced with A, G, I, L, T, M, or V; G267 replaced with A, I, L, S, T, M, or V; G269 replaced with A, I, L, S, T, M, or V; G270 replaced with A, I, L, S, T, M, or V; D271 replaced with E; K273 replaced with H, or R; M275 replaced with A, G, I, L, S, T, or V; D276 replaced with E; R277 replaced with H, or K; V278 replaced with A, G, I, L, S, T, or M; F280 replaced with W, or Y; W281 replaced with F, or Y; R282 replaced with H, or K; L283 replaced with A, G, I, S, T, M, or V; G284 replaced with A, I, L, S, T, M, or V; L285 replaced with A, G, I, S, T, M, or V; L286 replaced with A, G, I, S, T, M, or V; R287 replaced with H, or K; G288 replaced with A, I, L, S, T, M, or V; G290 replaced with A, I, L, S, T, M, or V; A291 replaced with G, I, L, S, T, M, or V; E292 replaced with D; D293 replaced with E; N294 replaced with Q; A295 replaced with G, I, L, S, T, M, or V; H296 replaced with K, or R; N297 replaced with Q; E298 replaced with D; I299 replaced with A, G, L, S, T, M, or V; L300 replaced with A, G, I, S, T, M, or V; S301 replaced with A, G, I, L, T, M, or V; N302 replaced with Q; A303 replaced with G, I, L, S, T, M, or V; D304 replaced with E; S305 replaced with A, G, I, L, T, M, or V; L306 replaced with A, G, I, S, T, M, or V; S307 replaced with A, G, I, L, T, M, or V; T308 replaced with A, G, I, L, S, M, or V; F309 replaced with W, or Y; V310 replaced with A, G, I, L, S, T, or M; S311 replaced with A, G, I, L, T, M, or V; E312 replaced with D; Q313 replaced with N; Q314 replaced with N; M315 replaced with A, G, I, L, S, T, or V; E316 replaced with D; S317 replaced with A, G, I, L, T, M, or V; Q318 replaced with N; E319 replaced with D; A321 replaced with G, I, L, S, T, M, or V; D322 replaced with E; L323 replaced with A, G, I, S, T, M, or V; T324 replaced with A, G, I, L, S, M, or V; G325 replaced with A, I, L, S, T, M, or V; V326 replaced with A, G, I, L, S, T, or M; T327 replaced with A, G, I, L, S, M, or V; V328 replaced with A, G, I, L, S, T, or M; Q329 replaced with N; S330 replaced with A, G, I, L, T, M, or V; G332 replaced with A, I, L, S, T, M, or V; E333 replaced with D; A334 replaced with G, I, L, S, T, M, or V; Q335 replaced with N; L337 replaced with A, G, I, S, T, M, or V; L338 replaced with A, G, I, S, T, M, or V; G339 replaced with A, I, L, S, T, M, or V; A341 replaced with G, I, L, S, T, M, or V; E342 replaced with D; A343 replaced with G, I, L, S, T, M, or V; E344 replaced with D; G345 replaced with A, I, L, S, T, M, or V; S346 replaced with A, G, I, L, T, M, or V; Q347 replaced with N; R348 replaced with H, or K; R349 replaced with H, or

K; R350 replaced with H, or K; L351 replaced with A, G, I, S, T, M, or V; L352 replaced with A, G, I, S, T, M, or V; V353 replaced with A, G, I, L, S, T, or M; A355 replaced with G, I, L, S, T, M, or V; N356 replaced with Q; G357 replaced with A, I, L, S, T, M, or V; A358 replaced with G, I, L, S, T, M, or V; D359 replaced with E; T361 replaced with A, G, I, L, S, M, or V; E362 replaced with D; T363 replaced with A, G, I, L, S, M, or V; L364 replaced with A, G, I, S, T, M, or V; M365 replaced with A, G, I, L, S, T, or V; L366 replaced with A, G, I, S, T, M, or V; F367 replaced with W, or Y; F368 replaced with W, or Y; D369 replaced with E; K370 replaced with H, or R; F371 replaced with W, or Y; A372 replaced with G, I, L, S, T, M, or V; N373 replaced with Q; I374 replaced with A, G, L, S, T, M, or V; V375 replaced with A, G, I, L, S, T, or M; F377 replaced with W, or Y; D378 replaced with E; S379 replaced with A, G, I, L, T, M, or V; W380 replaced with F, or Y; D381 replaced with E; Q382 replaced with N; L383 replaced with A, G, I, S, T, M, or V; M384 replaced with A, G, I, L, S, T, or V; R385 replaced with H, or K; Q386 replaced with N; L387 replaced with A, G, I, S, T, M, or V; D388 replaced with E; L389 replaced with A, G, I, S, T, M, or V; T390 replaced with A, G, I, L, S, M, or V; K391 replaced with H, or R; N392 replaced with Q; E393 replaced with D; I394 replaced with A, G, L, S, T, M, or V; D395 replaced with E; V396 replaced with A, G, I, L, S, T, or M; V397 replaced with A, G, I, L, S, T, or M; R398 replaced with H, or K; A399 replaced with G, I, L, S, T, M, or V; G400 replaced with A, I, L, S, T, M, or V; T401 replaced with A, G, I, L, S, M, or V; A402 replaced with G, I, L, S, T, M, or V; G403 replaced with A, I, L, S, T, M, or V; G405 replaced with A, I, L, S, T, M, or V; D406 replaced with E; A407 replaced with G, I, L, S, T, M, or V; L408 replaced with A, G, I, S, T, M, or V; Y409 replaced with F, or W; A410 replaced with G, I, L, S, T, M, or V; M411 replaced with A, G, I, L, S, T, or V; L412 replaced with A, G, I, S, T, M, or V; M413 replaced with A, G, I, L, S, T, or V; K414 replaced with H, or R; W415 replaced with F, or Y; V416 replaced with A, G, I, L, S, T, or M; N417 replaced with Q; K418 replaced with H, or R; T419 replaced with A, G, I, L, S, M, or V; G420 replaced with A, I, L, S, T, M, or V; R421 replaced with H, or K; N422 replaced with Q; A423 replaced with G, I, L, S, T, M, or V; S424 replaced with A, G, I, L, T, M, or V; I425 replaced with A, G, L, S, T, M, or V; H426 replaced with K, or R; T427 replaced with A, G, I, L, S, M, or V; L428 replaced with A, G, I, S, T, M, or V; L429 replaced with A, G, I, S, T, M, or V; D430 replaced with E; A431 replaced with G, I, L, S, T, M, or V; L432 replaced with A, G, I, S, T, M, or V; E433 replaced with D; R434 replaced with H, or K; M435 replaced

with A, G, I, L, S, T, or V; E436 replaced with D; E437 replaced with D; R438 replaced with H, or K; H439 replaced with K, or R; A440 replaced with G, I, L, S, T, M, or V; K441 replaced with H, or R; E442 replaced with D; K443 replaced with H, or R; I444 replaced with A, G, L, S, T, M, or V; Q445 replaced with N; D446 replaced with E; L447 replaced with A, G, I, S, T, M, or V; L448 replaced with A, G, I, S, T, M, or V; V449 replaced with A, G, I, L, S, T, or M; D450 replaced with E; S451 replaced with A, G, I, L, T, M, or V; G452 replaced with A, I, L, S, T, M, or V; K453 replaced with H, or R; F454 replaced with W, or Y; I455 replaced with A, G, L, S, T, M, or V; Y456 replaced with F, or W; L457 replaced with A, G, I, S, T, M, or V; E458 replaced with D; D459 replaced with E; G460 replaced with A, I, L, S, T, M, or V; T461 replaced with A, G, I, L, S, M, or V; G462 replaced with A, I, L, S, T, M, or V; S463 replaced with A, G, I, L, T, M, or V; A464 replaced with G, I, L, S, T, M, or V; V465 replaced with A, G, I, L, S, T, or M; S466 replaced with A, G, I, L, T, M, or V; L467 replaced with A, G, I, S, T, M, or V; and/or E468 replaced with D of SEQ ID NO:1.

**[0112]** In specific embodiments, the antibodies of the invention bind TR4 polypeptides or fragments or variants thereof (especially a fragment comprising or alternatively consisting of, the extracellular soluble domain of TR4), that contains any one or more of the following non-conservative mutations in TR4: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A2 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P3 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P4 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P5 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R7 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V8 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H9 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G11 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F13 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A15 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P18 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N19 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G21 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S22 replaced

with D, E, H, K, R, N, Q, F, W, Y, P, or C; A23 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G26 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T27 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E28 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T33 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P34 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S35 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K36 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V37 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W38 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S40 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G43 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R44 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E46 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P47 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R48 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R52 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P56 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S58 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M59 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G60 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q61 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; H62 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P64 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A66 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R67 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A68 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R69 replaced with D, E, A, G, I, L, S,



T, M, V, N, Q, F, W, Y, P, or C; A70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G71 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R72 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P74 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P76 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R77 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P78 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R80 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E81 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R85 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L86 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R87 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V88 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H89 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K90 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F92 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K93 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F94 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G98 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V99 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L101 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q102 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V104 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P105 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A108 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K112 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L113 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H114 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D115 replaced

with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q116 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S117 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I118 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q121 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q122 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; W123 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E124 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H125 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P127 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L128 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E130 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C132 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P133 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P134 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S136 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H137 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R138 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S139 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E140 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R141 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P142 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A144 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C145 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; N146 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R147 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C148 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E150 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G153 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y154 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N156 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A157 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S158 replaced with D, E, H, K, R, N,

Q, F, W, Y, P, or C; N159 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N160 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L161 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F162 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C164 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L165 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P166 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C167 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T168 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A169 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K171 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S172 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D173 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E174 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E175 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E176 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R177 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S178 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P179 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C180 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T181 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T182 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T183 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R184 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N185 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T186 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A187 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C188 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q189 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C190 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K191 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P192 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G193 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T194 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F195 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R196 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N197 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D198 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N199 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

A201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E202 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M203 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C204 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R205 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K206 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C207 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S208 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G210 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C211 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P212 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R213 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G214 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V216 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K217 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V218 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K219 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D220 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C221 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T222 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P223 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W224 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D226 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I227 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E228 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C229 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; V230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K232 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E233 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G235 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N236 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H238 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N239 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W241 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L244 replaced with D, E, H, K,

R, N, Q, F, W, Y, P, or C; V245 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T247 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L248 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P251 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L252 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L254 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L258 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V260 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C261 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C262 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C263 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I264 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G267 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; G269 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D271 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P272 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K273 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C274 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; M275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D276 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R277 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C279 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; F280 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W281 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R282 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L283 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R287 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P289 replaced with D, E, H, K, R, A, G, I, L,

S, T, M, V, N, Q, F, W, Y, or C; G290 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A291 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E292 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D293 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N294 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A295 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H296 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N297 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E298 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I299 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S301 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N302 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A303 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D304 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S305 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L306 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S307 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T308 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F309 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V310 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S311 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E312 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q313 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q314 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M315 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E316 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q318 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E319 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P320 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A321 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D322 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L323 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T324 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G325 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V326 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T327 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V328 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q329 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S330 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P331 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G332 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E333 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C;

A334 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q335 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C336 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L337 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L338 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G339 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P340 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A341 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E342 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A343 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E344 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G345 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S346 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q347 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R348 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R349 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R350 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L352 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V353 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P354 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A355 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N356 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G357 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A358 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D359 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P360 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T361 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E362 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T363 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L364 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M365 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L366 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F367 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F368 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D369 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K370 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F371 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A372 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N373 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I374 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V375 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P376 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F377 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D378 replaced

with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S379 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W380 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D381 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q382 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L383 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M384 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R385 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q386 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L387 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D388 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L389 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T390 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K391 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N392 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E393 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I394 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D395 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V396 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V397 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R398 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A399 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G400 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T401 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A402 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G403 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P404 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G405 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D406 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A407 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L408 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y409 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A410 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M411 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L412 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M413 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K414 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W415 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V416 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N417 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K418 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T419 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G420 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R421 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N422 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P,



or C; A423 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S424 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I425 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H426 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T427 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L428 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L429 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D430 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A431 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L432 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E433 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R434 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M435 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E436 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E437 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R438 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H439 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A440 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K441 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E442 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K443 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I444 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q445 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D446 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L447 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L448 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V449 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D450 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S451 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G452 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K453 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F454 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I455 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y456 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L457 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E458 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D459 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G460 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T461 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G462 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S463 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A464 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V465 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S466 replaced with D, E, H, K, R, N, Q, F, W, Y, P,

or C; L467 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or E468 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C of SEQ ID NO:1.

[0113] Amino acids in the TR4 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)). In preferred embodiments, antibodies of the present invention bind regions of TR4 that are essential for TR4 function. In other preferred embodiments, antibodies of the present invention bind regions of TR4 that are essential for TR4 function and inhibit or abolish TR4 function. In other preferred embodiments, antibodies of the present invention bind regions of TR4 that are essential for TR4 function and enhance TR4 function.

[0114] Additionally, protein engineering may be employed to improve or alter the characteristics of TR4 polypeptides. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may bind such modified TR4 polypeptides.

[0115] Non-naturally occurring variants of TR4 may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning; PCR mutagenesis, site directed mutagenesis (see e.g., Carter *et al.*, *Nucl. Acids Res.* 13:4331 (1986); and Zoller *et al.*, *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see e.g., Wells *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (see e.g., Wells *et al.*, *Philos. Trans. R. Soc. London SerA* 317:415 (1986)).

[0116] Thus, the invention also encompasses antibodies that bind TR4 derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to

generate TR4 polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the TR4 polypeptides and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the TR4 at the modified tripeptide sequence (see, e.g., Miyajimo et al., EMBO J 5(6):1193-1197). Additionally, one or more of the amino acid residues of TR4 polypeptides (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins.

**[0117]** The antibodies of the present invention also include antibodies that bind a polypeptide comprising, or alternatively, consisting of the polypeptide encoded by the deposited cDNA (the deposit having ATCC Accession Number 97853) including the leader; a polypeptide comprising, or alternatively, consisting of the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:1 including the leader; a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:1 minus the amino terminal methionine; a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:1 minus the leader; a polypeptide comprising, or alternatively, consisting of the TR4 extracellular domain; a polypeptide comprising, or alternatively, consisting of the TR4 cysteine rich domain; a polypeptide comprising, or alternatively, consisting of the TR4 transmembrane domain; a polypeptide comprising, or alternatively, consisting of the TR4 intracellular domain; a polypeptide comprising, or alternatively, consisting of the TR4 death domain; a polypeptide comprising, or alternatively, consisting of soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above (e.g., the polypeptide encoded by the deposited cDNA clone (the deposit having ATCC Accession Number 97853), the polypeptide of SEQ ID

NO:1, and portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

**[0118]** By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TR4 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the TR4 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

**[0119]** As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:1 or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

**[0120]** In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window

Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0121] The present application is also directed to antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to the TR4 polypeptide sequence set forth herein as  $n^1-m^1$ , and/or  $n^2-m^2$ . In preferred embodiments, the application is directed to antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific TR4 N- and C-terminal deletions recited herein.

[0122] In certain preferred embodiments, antibodies of the invention bind TR4 fusion proteins as described above wherein the TR4 portion of the fusion protein are those described as  $n^1-m^1$ , and/or  $n^2-m^2$  herein.

### TR7

[0123] In certain embodiments of the present invention, the antibodies of the present invention bind TR7 polypeptide, or fragments or variants thereof. The following section describes the TR7 polypeptides, fragments and variants that may be bound by the antibodies of the invention in more detail. The TR7 polypeptides, fragments and variants which may be bound by the antibodies of the invention are also described in, for example, International Publication Numbers WO98/41629, WO00/66156, and WO98/35986 which are herein incorporated by reference in their entireties.

[0124] In certain embodiments, the antibodies of the present invention immunospecifically bind TR7 polypeptide. An antibody that immunospecifically binds TR7 may, in some embodiments, bind fragments, variants (including species orthologs of TR7), multimers or modified forms of TR7. For example, an antibody immunospecific for TR7 may bind the TR7 moiety of a fusion protein comprising all or a portion of TR7.

[0125] TR7 proteins may be found as monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to antibodies that bind TR7 proteins found as monomers or as part of multimers. In specific embodiments, the TR7 polypeptides are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0126] Antibodies of the invention may bind TR7 homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only TR7 proteins of the invention (including TR7 fragments, variants, and fusion proteins, as described herein). These homomers may contain TR7 proteins having identical or different polypeptide

sequences. In a specific embodiment, a homomer of the invention is a multimer containing only TR7 proteins having an identical polypeptide sequence. In another specific embodiment, antibodies of the invention bind TR7 homomers containing TR7 proteins having different polypeptide sequences. In specific embodiments, antibodies of the invention bind a TR7 homodimer (e.g., containing TR7 proteins having identical or different polypeptide sequences) or a homotrimer (e.g., containing TR7 proteins having identical or different polypeptide sequences). In additional embodiments, antibodies of the invention bind at least a homodimer, at least a homotrimer, or at least a homotetramer of TR7.

[0127] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing polypeptide sequences that do not correspond to a polypeptide sequences encoded by the TR7 gene) in addition to the TR7 proteins of the invention. In a specific embodiment, antibodies of the invention bind a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the antibodies of the invention bind at least a homodimer, at least a homotrimer, or at least a homotetramer containing one or more TR7 polypeptides.

[0128] Multimers bound by one or more antibodies of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers bound by one or more antibodies of the invention, such as, for example, homodimers or homotrimers, are formed when TR7 proteins contact one another in solution. In another embodiment, heteromultimers bound by one or more antibodies of the invention, such as, for example, heterotrimers or heterotetramers, are formed when TR7 proteins contact antibodies to the TR7 polypeptides (including antibodies to the heterologous polypeptide sequence in a fusion protein) in solution. In other embodiments, multimers bound by one or more antibodies of the invention are formed by covalent associations with and/or between the TR7 proteins of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence recited in SEQ ID NO:3 or the polypeptide encoded by the deposited cDNA clone of ATCC Deposit 97920). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of

chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a TR7 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a TR7-Fc fusion protein (as described herein). In another specific example, covalent associations of fusion proteins are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety).

**[0129]** The multimers that may be bound by one or more antibodies of the invention may be generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers that may be bound by one or more antibodies of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, proteins that may be bound by one or more antibodies of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer that may be bound by one or more antibodies of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

**[0130]** Alternatively, multimers that may be bound by one or more antibodies of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers that may be bound by one or more



antibodies of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer that may be bound by one or more antibodies of the invention are generated by ligating a polynucleotide sequence encoding a TR7 polypeptide to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant TR7 polypeptides which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, two or more TR7 polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple TR7 polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology. In specific embodiments, antibodies of the invention bind proteins comprising multiple TR7 polypeptides separated by peptide linkers.

**[0131]** Another method for preparing multimer TR7 polypeptides involves use of TR7 polypeptides fused to a leucine zipper or isoleucine polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric TR7 proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble TR7 polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric TR7 is recovered from the culture supernatant using techniques known

in the art. In specific embodiments, antibodies of the invention bind TR7-leucine zipper fusion protein monomers and/or TR7-leucine zipper fusion protein multimers.

[0132] Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffer, *Science* 264:667, 1994; Banner et al., *Cell* 73:431, 1993). Thus, trimeric TR7 may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. In specific embodiments, antibodies of the invention bind TR7-leucine zipper fusion protein trimers.

[0133] Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric TR7. In specific embodiments, antibodies of the invention bind TR7- fusion protein monomers and/or TR7 fusion protein trimers.

[0134] Antibodies that bind TR7 receptor polypeptides may bind them as isolated polypeptides or in their naturally occurring state. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also, intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the TR7 polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). Thus, antibodies of the present invention may bind recombinantly produced TR7 receptor polypeptides. In a specific embodiment, antibodies of the present invention bind a TR7 receptor expressed on the surface of a cell comprising a polynucleotide encoding amino acids 1 to 411 of SEQ ID NO:3 operably associated with a regulatory sequence that controls gene expression.

[0135] Antibodies of the present invention may bind TR7 polypeptides or polypeptide fragments including polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:3, encoded by the cDNA contained in ATCC deposit Number 97920, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the ATCC deposit Number 97920, or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or

region, most preferably as a single continuous region. Antibodies of the present invention may bind polypeptide fragments, including, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 51, 52 to 78, 79 to 91, 92 to 111, 112 to 134, 135 to 151, 152 to 178, 179 to 180, 181 to 208, 209 to 218, 219 to 231, 232 to 251, 252 to 271, 272 to 291, 292 to 311, 312 to 323, 324 to 361, 362 to 391, 392 to 411 of SEQ ID NO:3. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Moreover, polypeptide fragments can be at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

**[0136]** Preferred polypeptide fragments of the present invention include a member selected from the group: a polypeptide comprising or alternatively, consisting of, the TR7 receptor extracellular domain (predicted to constitute amino acid residues from about 52 to about 184 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, both TR7 cysteine rich domains (both of which may be found in the protein fragment consisting of amino acid residues from about 84 to about 179 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, the TR7 cysteine rich domain consisting of amino acid residues from about 84 to about 131 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, the TR7 cysteine rich domain consisting of amino acid residues from about 132 to about 179 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, the TR7 receptor transmembrane domain (predicted to constitute amino acid residues from about 185 to about 208 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, fragment of the predicted mature TR7 polypeptide, wherein the fragment has a TR7 functional activity (e.g., antigenic activity or biological activity); a polypeptide comprising or alternatively, consisting of, the TR7 receptor intracellular domain (predicted to constitute amino acid residues from about 209 to about 411 in SEQ ID NO:3); a polypeptide comprising or alternatively, consisting of, the TR7 receptor extracellular and intracellular domains with all or part of the transmembrane domain deleted; a polypeptide comprising, or alternatively consisting of, the TR7 receptor death domain (predicted to constitute amino acid residues from about 324 to about 391 in SEQ ID NO:3); and a polypeptide comprising, or alternatively, consisting of, one, two, three, four or more, epitope bearing

portions of the TR7 receptor protein. In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively, consist of, any combination of 1, 2, 3, 4, 5, 6, 7, or all 8 of the above members. As above, with the leader sequence, the amino acid residues constituting the TR7 receptor extracellular, transmembrane and intracellular domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain. Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention.

[0137] As discussed above, it is believed that one or both of the extracellular cysteine rich motifs of TR7 is important for interactions between TR7 and its ligands (e.g., TRAIL). Accordingly, in highly preferred embodiments, antibodies of the present invention bind TR7 polypeptide fragments comprising, or alternatively consisting of, amino acid residues 84 to 131, and/or 132 to 179 of SEQ ID NO:3. In another highly preferred embodiment, antibodies of the present invention bind TR7 polypeptides comprising, or alternatively consisting of, both of the extracellular cysteine rich motifs (amino acid residues 84 to 179 of SEQ ID NO:3.) In another preferred embodiment, antibodies of the present invention bind TR7 polypeptides comprising, or alternatively consisting the extracellular soluble domain of TR7 (amino acid residues 52 to 184 of SEQ ID NO:2.) In other highly preferred embodiments, the antibodies of the invention that bind all or a portion of the extracellular soluble domain of TR7 (e.g., one or both cysteine rich domains) agonize the TR7 receptor.

[0138] In other highly preferred embodiments, the antibodies of the invention that bind all or a portion of the extracellular soluble domain of TR7 (e.g., one or both cysteine rich domains) induce cell death of the cell expressing the TR7 receptor.

[0139] Antibodies of the invention may also bind fragments comprising, or alternatively, consisting of structural or functional attributes of TR7. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., regions of polypeptides consisting of amino acid residues having an antigenic index of or equal to

greater than 1.5, as identified using the default parameters of the Jameson-Wolf program) of TR7. Certain preferred regions are those disclosed in Table 4 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence of SEQ ID NO:3, such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic regions and Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

**[0140]** The data representing the structural or functional attributes of TR7 set forth in Table 4, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. Column I represents the results of a Garnier-Robson analysis of alpha helical regions; Column II represents the results of a Chou-Fasman analysis of alpha helical regions; Column III represents the results of a Garnier Robson analysis of beta sheet regions; Column IV represents the results of a Chou-Fasman analysis of beta sheet regions; Column V represents the results of a Garnier Robson analysis of turn regions; Column VI represents the results of a Chou-Fasman analysis of turn regions; Column VII represents the results of a Garnier Robson analysis of coil regions; Column VIII represents a Kyte-Doolittle hydrophilicity plot; Column IX represents a Hopp-Woods hydrophobicity plot; Column X represents the results of an Eisenberg analysis of alpha amphipathic regions; Column XI represents the results of an Eisenberg analysis of beta amphipathic regions; Column XII represents the results of a Karplus-Schultz analysis of flexible regions; Column XIII represents the Jameson-Wolf antigenic index score; and Column XIV represents the Emini surface probability plot.

**[0141]** In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table 4 can be used to determine regions of TR7 which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. The columns in Table 4 present the result of different analyses of the TR7 protein sequence.

**[0142]** The above-mentioned preferred regions set out in Table 4 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in SEQ ID NO:3. As set out in Table 4, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Jameson-Wolf regions of high antigenic index and Emini surface-forming regions. Preferably, antibodies of the present invention bind TR7 polypeptides or TR7 polypeptide fragments and variants comprising regions of TR7 that combine several structural features, such as several (e.g., 1, 2, 3 , or 4) of the same or different region features set out above and in Table 4.

Table 4

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	A	.	.	.	.	.	.	1.11	-0.70	.	*	.	1.29	2.18
Glu	2	A	.	.	.	.	.	.	1.50	-0.70	.	*	.	1.63	1.69
Gln	3	A	.	.	.	.	T	.	1.89	-0.73	.	*	.	2.17	2.28
Arg	4	.	.	.	.	T	T	.	1.69	-0.76	.	*	.	2.91	3.71
Gly	5	.	.	.	.	T	T	.	1.87	-0.87	.	*	F	3.40	2.17
Gln	6	.	.	.	.	T	T	.	1.88	-0.44	.	*	F	2.76	1.93
Asn	7	.	.	.	.	.	.	C	1.29	-0.34	.	*	F	1.87	1.00
Ala	8	.	.	.	.	.	.	C	0.99	0.16	.	.	F	1.08	1.02
Pro	9	.	.	.	.	.	.	C	0.53	0.11	.	*	.	0.44	0.79
Ala	10	A	.	.	.	.	.	.	0.29	0.14	.	*	.	-0.10	0.48
Ala	11	A	.	.	.	.	T	.	0.40	0.24	.	.	.	0.10	0.48
Ser	12	A	.	.	.	.	T	.	0.44	-0.26	.	*	F	0.85	0.61
Gly	13	A	.	.	.	.	T	.	1.14	-0.69	.	*	F	1.30	1.22
Ala	14	A	.	.	.	.	T	.	1.32	-1.19	.	*	F	1.30	2.36
Arg	15	A	.	.	.	T	.	.	1.57	-1.19	.	*	F	1.50	2.39
Lys	16	.	.	.	.	T	.	.	1.94	-1.14	.	.	F	1.50	2.39
Arg	17	.	.	.	.	T	.	.	1.90	-1.14	.	*	F	1.80	3.66
His	18	.	.	.	.	.	.	C	2.03	-1.21	*	*	F	1.90	1.85
Gly	19	.	.	.	.	.	T	C	2.73	-0.79	*	*	F	2.40	1.43
Pro	20	.	.	.	.	.	T	C	2.62	-0.79	*	*	F	2.70	1.43
Gly	21	.	.	.	.	.	T	C	1.99	-0.79	*	.	F	3.00	1.82
Pro	22	.	.	.	.	.	T	C	1.99	-0.79	.	*	F	2.70	1.86
Arg	23	.	A	.	.	.	.	C	1.68	-1.21	*	.	F	2.30	2.35
Glu	24	.	A	B	.	.	.	.	1.43	-1.21	*	.	F	2.10	2.35
Ala	25	.	A	.	.	T	.	.	1.76	-1.14	*	.	F	2.50	1.54
Arg	26	.	A	.	.	T	.	.	1.89	-1.57	*	.	F	2.50	1.54
Gly	27	.	.	.	.	T	.	.	1.76	-1.14	*	.	F	3.00	1.37
Ala	28	.	.	.	.	T	.	C	1.43	-0.71	*	*	F	2.70	1.35
Arg	29	.	.	.	.	.	T	C	1.54	-0.79	*	*	F	2.66	1.06
Pro	30	.	.	.	.	.	T	C	1.28	-0.79	*	*	F	2.62	2.10
Gly	31	.	.	.	.	.	T	C	0.96	-0.57	*	*	F	2.58	1.54
Pro	32	.	.	.	.	.	T	C	1.34	-0.64	*	*	F	2.54	1.22
Arg	33	.	.	.	.	.	.	C	1.62	-0.64	*	*	F	2.60	1.58
Val	34	.	.	.	.	.	.	C	0.70	-0.59	*	*	F	2.34	2.30
Pro	35	.	.	B	.	.	.	.	0.06	-0.33	*	*	F	1.58	1.23
Lys	36	.	.	B	B	.	.	.	-0.41	-0.11	*	.	F	0.97	0.46
Thr	37	.	.	B	B	.	.	.	-1.06	0.57	*	*	F	-0.19	0.52
Leu	38	.	.	B	B	.	.	.	-2.02	0.57	*	*	.	-0.60	0.25
Val	39	.	.	B	B	.	.	.	-1.76	0.79	.	.	.	-0.60	0.09
Leu	40	A	.	.	B	.	.	.	-2.13	1.29	.	.	.	-0.60	0.06
Val	41	A	.	.	B	.	.	.	-3.03	1.30	.	.	.	-0.60	0.08
Val	42	A	.	.	B	.	.	.	-3.53	1.26	.	.	.	-0.60	0.08
Ala	43	A	.	.	B	.	.	.	-3.53	1.30	.	.	.	-0.60	0.08
Ala	44	A	.	.	B	.	.	.	-3.49	1.30	.	.	.	-0.60	0.09
Val	45	A	.	.	B	.	.	.	-3.53	1.34	.	.	.	-0.60	0.10
Leu	46	A	.	.	B	.	.	.	-2.98	1.34	.	.	.	-0.60	0.07
Leu	47	A	.	.	B	.	.	.	-2.71	1.23	.	.	.	-0.60	0.09
Leu	48	A	.	.	B	.	.	.	-2.12	1.23	.	.	.	-0.60	0.13
Val	49	A	.	.	B	.	.	.	-1.83	0.59	.	.	.	-0.60	0.27
Ser	50	A	.	.	B	.	.	.	-1.57	0.29	.	*	.	-0.30	0.44

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ala	51	A	A	.	.	.	.	.	-1.57	0.10	.	.	.	-0.30	0.54
Glu	52	A	A	.	.	.	.	.	-1.64	0.10	.	.	.	-0.30	0.60
Ser	53	A	A	.	B	.	.	.	-1.14	0.14	.	.	.	-0.30	0.31
Ala	54	A	A	.	B	.	.	.	-0.29	0.24	.	.	.	-0.30	0.45
Leu	55	A	A	.	B	.	.	.	0.01	0.14	.	.	.	-0.30	0.45
Ile	56	A	A	.	B	.	.	.	0.60	0.54	.	.	.	-0.60	0.58
Thr	57	A	A	.	B	.	.	.	-0.21	0.16	.	.	F	-0.15	0.96
Gln	58	A	A	.	B	.	.	.	-0.50	0.34	.	.	F	-0.15	0.96
Gln	59	A	A	.	B	.	.	.	-0.12	0.16	.	.	F	0.00	1.38
Asp	60	.	A	.	B	T	.	.	0.69	-0.10	.	.	F	1.00	1.48
Leu	61	.	A	.	.	.	.	C	1.58	-0.19	.	*	F	0.80	1.48
Ala	62	.	A	.	.	.	.	C	2.00	-0.19	.	*	F	0.80	1.48
Pro	63	.	A	.	.	.	.	C	1.41	-0.59	.	*	F	1.10	1.73
Gln	64	.	A	.	.	T	.	.	0.82	-0.09	.	*	F	1.00	2.13
Gln	65	A	A	.	.	.	.	.	0.61	-0.27	.	*	F	0.60	2.13
Arg	66	A	A	.	.	.	.	.	1.42	-0.34	.	*	F	0.60	2.13
Ala	67	A	A	.	.	.	.	.	2.01	-0.37	.	*	F	0.94	2.13
Ala	68	A	A	.	.	.	.	.	2.27	-0.37	*	*	F	1.28	2.13
Pro	69	A	A	.	.	.	.	.	2.38	-0.77	*	*	F	1.92	2.17
Gln	70	.	A	.	.	T	.	.	2.08	-0.77	*	.	F	2.66	4.21
Gln	71	.	.	.	.	T	T	.	1.67	-0.89	*	*	F	3.40	5.58
Lys	72	.	.	.	.	T	T	.	2.04	-1.00	.	.	F	3.06	4.84
Arg	73	.	.	.	.	T	T	.	2.33	-1.00	.	.	F	2.97	4.32
Ser	74	.	.	.	.	.	T	C	2.54	-1.01	.	.	F	2.68	3.34
Ser	75	.	.	.	.	.	T	C	2.20	-1.41	.	.	F	2.59	2.89
Pro	76	.	.	.	.	T	T	.	1.39	-0.99	.	.	F	2.70	1.46
Ser	77	.	.	.	.	T	T	.	0.68	-0.30	.	.	F	2.50	0.90
Glu	78	.	.	.	.	T	T	.	0.36	-0.11	.	*	F	2.25	0.36
Gly	79	.	.	.	.	T	.	.	0.44	-0.07	.	.	F	1.80	0.36
Leu	80	.	.	.	.	T	.	.	0.40	-0.07	.	.	F	1.55	0.42
Cys	81	.	.	.	.	.	.	C	0.58	-0.03	.	.	.	0.95	0.24
Pro	82	.	.	.	.	.	T	C	0.84	0.47	*	.	F	0.15	0.33
Pro	83	.	.	.	.	T	T	.	-0.04	0.54	*	.	F	0.35	0.54
Gly	84	.	.	.	.	T	T	.	0.00	0.54	*	.	.	0.20	0.70
His	85	.	.	.	.	.	T	C	0.81	0.36	*	.	.	0.30	0.61
His	86	.	.	.	.	.	.	C	1.48	-0.07	*	.	.	0.70	0.68
Ile	87	.	.	.	.	.	.	C	1.34	-0.50	*	*	.	1.19	1.15
Ser	88	.	.	.	.	.	.	C	1.67	-0.50	*	*	F	1.53	0.84
Glu	89	.	.	.	.	T	.	.	2.01	-1.00	*	*	F	2.52	1.21
Asp	90	.	.	.	.	T	.	.	1.38	-1.50	*	*	F	2.86	2.88
Gly	91	.	.	.	.	T	T	.	0.52	-1.61	*	*	F	3.40	1.15
Arg	92	.	.	.	.	T	T	.	1.11	-1.31	*	*	F	2.91	0.47
Asp	93	.	.	.	.	T	T	.	0.74	-0.93	.	*	F	2.57	0.37
Cys	94	.	.	.	.	T	T	.	0.79	-0.36	.	*	.	1.78	0.20
Ile	95	.	.	.	.	T	.	.	0.54	-0.79	.	*	.	1.54	0.21
Ser	96	.	.	.	.	T	.	.	0.54	-0.03	.	*	.	1.18	0.19
Cys	97	.	.	.	.	T	T	.	0.43	0.40	.	*	.	0.76	0.36
Lys	98	.	.	.	.	T	T	.	0.43	0.23	.	.	.	1.34	0.88
Tyr	99	.	.	.	.	T	T	.	0.86	-0.46	.	*	F	2.52	1.10
Gly	100	.	.	.	.	T	T	.	1.44	-0.09	.	*	F	2.80	3.22



Table 4

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gln	101	.	.	.	.	T	T	.	1.43	-0.27	*	.	F	2.52	2.16
Asp	102	.	.	.	.	T	T	.	2.07	0.21	*	*	F	1.64	1.99
Tyr	103	.	.	.	.	T	T	.	1.73	-0.04	*	*	F	1.96	2.73
Ser	104	.	.	.	.	T	T	.	1.98	0.44	*	.	F	0.78	1.66
Thr	105	.	.	.	.	T	.	.	2.32	0.44	*	.	F	0.30	1.60
His	106	.	.	.	.	T	.	.	1.51	0.44	*	.	.	0.15	1.70
Trp	107	.	.	.	.	T	T	.	0.70	0.37	*	.	.	0.65	1.05
Asn	108	.	.	.	.	T	T	.	0.24	0.67	.	.	.	0.20	0.60
Asp	109	.	.	.	.	T	T	.	-0.12	0.97	*	.	.	0.20	0.38
Leu	110	A	.	.	.	.	T	.	-0.62	1.04	*	*	.	-0.20	0.19
Leu	111	.	.	.	B	T	.	.	-0.48	0.81	*	*	.	-0.20	0.10
Phe	112	.	.	.	B	T	.	.	-0.86	0.41	*	*	.	-0.20	0.12
Cys	113	.	.	.	B	T	.	.	-1.17	0.99	*	*	.	-0.20	0.08
Leu	114	.	.	.	B	T	.	.	-1.06	0.79	.	*	.	-0.20	0.13
Arg	115	.	.	.	B	T	.	.	-0.91	0.10	.	*	.	0.10	0.30
Cys	116	.	.	.	B	T	.	.	-0.10	-0.11	.	.	.	0.70	0.30
Thr	117	.	.	.	B	T	.	.	0.30	-0.69	.	*	.	1.00	0.61
Arg	118	.	.	.	B	T	.	.	0.62	-0.99	.	.	F	1.49	0.42
Cys	119	.	.	.	.	T	T	.	1.43	-0.56	*	.	F	2.23	0.77
Asp	120	.	.	.	.	T	T	.	0.47	-1.13	*	.	F	2.57	0.92
Ser	121	.	.	.	.	T	T	.	1.13	-0.97	.	*	F	2.91	0.35
Gly	122	.	.	.	.	T	T	.	0.63	-0.97	.	*	F	3.40	1.13
Glu	123	.	A	.	.	T	.	.	0.22	-0.86	.	*	F	2.51	0.56
Val	124	A	A	.	.	.	.	.	0.68	-0.47	.	*	F	1.47	0.56
Glu	125	.	A	.	.	T	.	.	0.01	-0.43	.	*	.	1.38	0.87
Leu	126	.	A	.	.	T	.	.	0.00	-0.29	.	*	.	1.04	0.27
Ser	127	.	.	.	.	.	T	C	0.03	0.20	.	*	F	0.45	0.52
Pro	128	.	.	.	.	T	T	.	-0.28	0.04	.	*	F	0.93	0.44
Cys	129	.	.	.	.	T	T	.	0.69	0.53	.	*	F	0.91	0.77
Thr	130	.	.	.	.	T	T	.	0.69	-0.16	.	*	F	2.24	1.12
Thr	131	.	.	.	.	T	.	.	1.19	-0.14	.	*	F	2.32	1.16
Thr	132	.	.	.	.	T	T	.	0.63	-0.09	.	*	F	2.80	3.13
Arg	133	.	.	.	.	T	T	.	0.18	-0.01	.	.	F	2.52	1.61
Asn	134	.	.	.	.	T	T	.	0.84	0.07	.	.	F	1.49	0.60
Thr	135	.	.	.	.	T	T	.	0.49	-0.01	.	.	F	1.81	0.72
Val	136	.	.	.	.	T	.	C	0.80	0.07	*	.	.	0.58	0.20
Cys	137	.	A	.	.	T	.	.	1.11	0.07	*	.	.	0.10	0.21
Gln	138	.	A	B	.	.	.	.	0.66	-0.33	*	.	.	0.30	0.25
Cys	139	.	A	.	.	T	.	.	0.34	-0.39	.	.	.	0.70	0.34
Glu	140	A	A	.	.	.	.	.	-0.04	-0.54	*	*	F	0.75	0.91
Glu	141	A	A	.	.	.	.	.	0.92	-0.33	*	*	F	0.45	0.46
Gly	142	.	A	.	.	T	.	.	1.59	-0.73	.	*	F	1.30	1.67
Thr	143	A	A	.	.	.	.	.	1.59	-1.30	.	*	F	0.90	1.67
Phe	144	A	A	.	.	.	.	.	2.26	-1.30	.	*	F	0.90	1.67
Arg	145	A	A	.	.	.	.	.	1.96	-1.30	.	*	F	0.90	2.81
Glu	146	A	A	.	.	.	.	.	1.74	-1.34	.	*	F	0.90	2.61
Glu	147	A	A	.	.	.	.	.	2.09	-1.40	.	*	F	0.90	4.66
Asp	148	A	A	.	.	.	.	.	1.80	-2.19	.	*	F	0.90	4.12
Ser	149	A	.	.	.	.	T	.	1.83	-1.57	.	*	F	1.30	2.35
Pro	150	A	.	.	.	.	T	.	1.83	-1.00	.	.	F	1.15	0.73

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Glu	151	A	.	.	.	.	T	.	1.88	-1.00	*	.	F	1.15	0.85
Met	152	A	.	.	.	.	T	.	1.21	-1.00	*	*	.	1.49	1.28
Cys	153	A	.	.	.	.	T	.	1.32	-0.81	*	*	.	1.68	0.44
Arg	154	A	.	.	.	.	T	.	1.31	-1.24	*	.	.	2.02	0.50
Lys	155	.	.	.	.	T	T	.	1.18	-0.76	*	*	F	2.91	0.73
Cys	156	.	.	.	.	T	T	.	0.51	-0.94	*	.	F	3.40	1.35
Arg	157	.	.	.	.	T	.	.	0.90	-0.94	*	.	F	2.71	0.37
Thr	158	.	.	.	.	T	.	.	1.68	-0.51	*	.	F	2.37	0.28
Gly	159	.	.	.	.	T	.	.	1.22	-0.51	*	.	F	2.43	1.04
Cys	160	.	.	.	.	.	T	C	0.58	-0.66	.	*	F	2.19	0.53
Pro	161	.	.	.	.	T	T	.	0.39	-0.04	.	*	F	2.00	0.36
Arg	162	.	.	.	.	T	T	.	0.32	0.11	.	*	F	1.65	0.27
Gly	163	.	.	.	.	T	T	.	-0.22	-0.31	*	*	.	2.50	1.01
Met	164	.	.	B	B	.	.	.	-0.22	-0.24	*	*	.	1.30	0.48
Val	165	.	.	B	B	.	.	.	0.44	-0.24	*	*	.	1.30	0.24
Lys	166	.	.	B	B	.	.	.	-0.01	-0.24	*	*	.	1.30	0.41
Val	167	.	.	B	.	.	T	.	-0.43	-0.10	*	*	F	1.85	0.22
Gly	168	.	.	.	.	T	T	.	-0.30	-0.23	.	.	F	2.25	0.44
Asp	169	.	.	.	.	T	T	.	0.01	-0.44	.	.	F	2.50	0.34
Cys	170	.	.	.	.	T	T	.	0.57	0.47	.	*	F	1.35	0.48
Thr	171	.	.	.	.	.	T	C	0.52	0.21	.	*	F	1.20	0.65
Pro	172	.	.	.	.	T	T	.	0.49	-0.21	.	*	F	1.75	0.65
Trp	173	.	.	.	.	T	T	.	0.83	0.47	.	*	F	0.60	0.84
Ser	174	A	.	.	.	.	T	.	0.17	-0.10	.	*	F	1.00	1.01
Asp	175	A	A	.	.	.	.	.	-0.02	-0.01	.	.	F	0.45	0.35
Ile	176	A	A	.	.	.	.	.	0.26	0.20	*	*	.	-0.30	0.25
Glu	177	A	A	.	.	.	.	.	0.51	-0.21	*	.	.	0.30	0.25
Cys	178	A	A	.	.	.	.	.	0.80	-0.60	*	.	.	0.60	0.30
Val	179	A	A	.	.	.	.	.	0.80	-0.60	*	*	.	0.60	0.74
His	180	A	A	.	.	.	.	.	0.46	-0.90	.	*	.	0.60	0.58
Lys	181	A	A	.	.	.	.	.	0.46	-0.47	*	.	F	0.60	1.06
Glu	182	A	.	.	.	.	T	.	-0.43	-0.36	*	.	F	1.00	1.00
Ser	183	A	.	.	.	.	T	.	-0.66	-0.31	.	.	F	0.85	0.52
Gly	184	A	.	.	.	T	T	.	-0.14	-0.13	.	.	F	1.25	0.18
Ile	185	A	.	.	.	.	T	.	-0.97	0.30	.	.	.	0.10	0.10
Ile	186	.	.	B	B	.	.	.	-1.32	0.94	.	*	.	-0.60	0.06
Ile	187	.	.	B	B	.	.	.	-2.18	1.04	.	.	.	-0.60	0.08
Gly	188	.	.	B	B	.	.	.	-2.47	1.26	.	*	.	-0.60	0.09
Val	189	.	.	B	B	.	.	.	-2.71	1.07	.	.	.	-0.60	0.13
Thr	190	A	.	.	B	.	.	.	-2.68	0.89	.	*	.	-0.60	0.18
Val	191	A	.	.	B	.	.	.	-2.64	0.84	.	.	.	-0.60	0.14
Ala	192	A	.	.	B	.	.	.	-2.57	1.06	.	*	.	-0.60	0.14
Ala	193	A	.	.	B	.	.	.	-3.11	1.10	.	.	.	-0.60	0.08
Val	194	A	.	.	B	.	.	.	-3.11	1.30	.	.	.	-0.60	0.07
Val	195	A	.	.	B	.	.	.	-3.39	1.30	.	.	.	-0.60	0.05
Leu	196	A	.	.	B	.	.	.	-3.39	1.30	.	.	.	-0.60	0.05
Ile	197	A	.	.	B	.	.	.	-3.50	1.44	.	.	.	-0.60	0.05
Val	198	A	.	.	B	.	.	.	-3.77	1.59	.	.	.	-0.60	0.06
Ala	199	A	.	.	B	.	.	.	-3.58	1.59	.	.	.	-0.60	0.06
Val	200	A	.	.	B	.	.	.	-2.68	1.47	.	.	.	-0.60	0.04

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Phe	201	A	.	.	B	.	.	.	-2.17	0.79	.	.	.	-0.60	0.12
Val	202	A	.	.	B	.	.	.	-2.09	0.53	.	.	.	-0.60	0.16
Cys	203	A	.	.	.	.	T	.	-2.04	0.71	.	.	.	-0.20	0.17
Lys	204	A	.	.	.	.	T	.	-1.74	0.76	.	.	.	-0.20	0.17
Ser	205	A	.	.	.	.	T	.	-0.84	0.89	.	.	.	-0.20	0.24
Leu	206	A	.	.	.	.	T	.	-0.10	0.24	.	.	.	0.10	0.88
Leu	207	A	A	.	.	.	.	.	-0.10	-0.33	.	.	.	0.30	0.88
Trp	208	A	A	.	.	.	.	.	-0.24	0.31	.	.	.	-0.30	0.49
Lys	209	A	A	.	.	.	.	.	-0.50	0.61	.	.	.	-0.60	0.49
Lys	210	A	A	.	.	.	.	.	-0.44	0.36	*	.	.	-0.30	0.91
Val	211	A	A	.	.	.	.	.	-0.44	0.43	*	*	.	-0.45	1.36
Leu	212	.	A	B	.	.	.	.	0.41	0.20	*	*	.	-0.30	0.56
Pro	213	.	A	B	.	.	.	.	0.36	0.20	*	.	.	-0.30	0.56
Tyr	214	.	.	.	B	T	.	.	-0.58	0.63	*	.	.	-0.20	0.75
Leu	215	.	.	.	B	T	.	.	-1.29	0.67	*	*	.	-0.20	0.64
Lys	216	.	.	.	B	T	.	.	-0.73	0.56	*	.	.	-0.20	0.22
Gly	217	.	.	B	B	.	.	.	-0.27	0.51	*	.	.	-0.60	0.19
Ile	218	.	.	B	B	.	.	.	-0.40	0.19	*	.	.	-0.30	0.23
Cys	219	.	.	B	.	.	T	.	-0.50	-0.07	*	.	.	0.70	0.11
Ser	220	.	.	.	.	T	T	.	-0.03	0.36	.	*	F	0.65	0.11
Gly	221	.	.	.	.	T	T	.	-0.08	0.36	.	.	F	0.65	0.16
Gly	222	.	.	.	.	T	T	.	0.06	-0.33	.	.	F	1.25	0.49
Gly	223	.	.	.	.	.	.	C	0.94	-0.47	.	.	F	0.85	0.57
Gly	224	.	.	.	.	.	.	C	1.72	-0.86	*	.	F	1.15	0.99
Asp	225	.	.	.	.	.	T	C	1.17	-1.29	.	*	F	1.50	1.97
Pro	226	.	.	.	.	.	T	C	1.51	-1.07	*	.	F	1.84	1.47
Glu	227	.	.	B	.	.	T	.	1.97	-1.50	*	.	F	1.98	2.49
Arg	228	.	.	B	.	.	T	.	2.01	-1.93	*	.	F	2.32	2.92
Val	229	.	.	.	.	T	.	.	2.06	-1.54	*	.	F	2.86	2.53
Asp	230	.	.	.	.	T	T	.	2.06	-1.59	*	.	F	3.40	1.96
Arg	231	.	.	.	.	T	T	.	2.38	-1.19	*	*	F	3.06	1.73
Ser	232	.	.	.	.	T	T	.	2.17	-1.19	*	.	F	2.72	4.57
Ser	233	.	.	.	.	T	T	.	1.71	-1.40	*	*	F	2.72	4.23
Gln	234	.	.	.	.	.	.	C	1.98	-0.97	*	*	F	2.32	2.14
Arg	235	.	.	.	.	.	T	C	1.98	-0.47	*	*	F	2.22	1.61
Pro	236	.	.	.	.	.	T	C	1.87	-0.86	*	*	F	2.86	2.08
Gly	237	.	.	.	.	T	T	.	2.17	-1.24	.	*	F	3.40	2.01
Ala	238	.	.	.	.	.	T	C	1.61	-1.24	.	*	F	2.86	1.65
Glu	239	A	.	.	.	.	.	.	0.80	-0.60	.	*	F	1.97	0.79
Asp	240	A	.	.	.	.	.	.	0.69	-0.34	.	*	F	1.33	0.66
Asn	241	A	.	.	.	.	.	.	0.90	-0.37	*	.	.	0.99	1.05
Val	242	A	.	.	.	.	.	.	0.36	-0.87	*	.	.	0.95	1.05
Leu	243	A	.	.	.	.	.	.	0.09	-0.19	*	.	.	0.50	0.44
Asn	244	A	.	.	B	.	.	.	-0.21	0.46	*	.	.	-0.60	0.20
Glu	245	A	.	.	B	.	.	.	-1.10	0.44	*	.	.	-0.60	0.37
Ile	246	A	.	.	B	.	.	.	-1.91	0.49	*	.	.	-0.60	0.31
Val	247	A	.	.	B	.	.	.	-1.06	0.49	*	.	.	-0.60	0.16
Ser	248	.	.	B	B	.	.	.	-0.46	0.49	*	.	.	-0.60	0.16
Ile	249	.	.	B	B	.	.	.	-0.77	0.91	*	.	.	-0.60	0.35
Leu	250	.	.	.	B	.	.	C	-0.77	0.71	.	.	.	-0.40	0.69

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gln	251	.	.	.	.	.	T	C	-0.73	0.47	.	.	F	0.15	0.89
Pro	252	.	.	.	.	.	T	C	-0.09	0.73	.	.	F	0.15	0.94
Thr	253	.	.	.	.	.	T	C	0.21	0.47	.	.	F	0.30	1.76
Gln	254	.	.	.	.	.	T	C	1.10	-0.21	.	.	F	1.20	1.76
Val	255	.	A	.	.	.	.	C	1.91	-0.21	.	.	F	0.80	1.97
Pro	256	.	A	.	.	.	.	C	1.31	-0.64	.	.	F	1.10	2.37
Glu	257	A	A	.	.	.	.	.	1.52	-0.51	.	*	F	0.90	1.35
Gln	258	A	A	.	.	.	.	.	0.98	-0.91	.	*	F	0.90	3.16
Glu	259	A	A	.	.	.	.	.	0.98	-0.91	.	*	F	0.90	1.51
Met	260	A	A	.	.	.	.	.	1.83	-0.94	.	*	F	0.90	1.51
Glu	261	A	A	.	.	.	.	.	1.83	-0.94	.	*	.	0.75	1.51
Val	262	A	A	.	.	.	.	.	1.24	-0.91	.	*	F	0.90	1.35
Gln	263	A	A	.	.	.	.	.	1.24	-0.41	.	*	F	0.60	1.38
Glu	264	A	A	.	.	.	.	.	1.03	-1.03	.	*	F	0.90	1.38
Pro	265	A	A	.	.	.	.	.	1.32	-0.60	.	*	F	1.18	2.88
Ala	266	A	A	.	.	.	.	.	0.98	-0.76	.	*	F	1.46	2.40
Glu	267	A	.	.	.	.	T	.	0.98	-0.73	.	*	F	2.14	1.37
Pro	268	A	.	.	.	.	T	.	0.98	-0.09	.	.	F	1.97	0.66
Thr	269	.	.	.	.	T	T	.	0.38	-0.11	.	.	F	2.80	1.05
Gly	270	A	.	.	.	.	T	.	-0.22	0.00	.	.	F	1.37	0.60
Val	271	A	.	.	.	.	.	.	0.07	0.69	.	.	.	0.44	0.32
Asn	272	.	.	B	.	.	.	.	-0.14	0.64	.	.	.	0.16	0.30
Met	273	.	.	B	.	.	.	.	-0.28	0.59	.	.	.	0.18	0.46
Leu	274	.	.	.	.	.	.	C	0.03	0.59	.	.	.	0.40	0.62
Ser	275	.	.	.	.	.	T	C	0.08	-0.06	.	.	F	1.95	0.66
Pro	276	.	.	.	.	.	T	C	0.93	-0.07	.	.	F	2.25	0.90
Gly	277	.	.	.	.	.	T	C	0.90	-0.69	.	.	F	3.00	1.89
Glu	278	A	.	.	.	.	T	.	0.69	-0.87	.	.	F	2.50	1.92
Ser	279	A	A	.	.	.	.	.	0.69	-0.57	.	.	F	1.80	1.02
Glu	280	A	A	.	.	.	.	.	0.99	-0.31	.	.	F	1.05	0.85
His	281	A	A	.	.	.	.	.	0.99	-0.74	.	.	F	1.05	0.85
Leu	282	A	A	.	.	.	.	.	0.74	-0.31	.	.	.	0.30	0.98
Leu	283	A	A	.	.	.	.	.	0.74	-0.20	.	.	.	0.30	0.57
Glu	284	A	A	.	.	.	.	.	0.46	-0.20	.	.	F	0.45	0.73
Pro	285	A	A	.	.	.	.	.	0.46	-0.20	.	.	F	0.45	0.89
Ala	286	A	A	.	.	.	.	.	0.60	-0.89	.	.	F	0.90	1.88
Glu	287	A	A	.	.	.	.	.	1.11	-1.57	.	.	F	0.90	2.13
Ala	288	A	A	.	.	.	.	.	1.92	-1.19	.	.	F	0.90	1.84
Glu	289	A	A	.	.	.	.	.	2.03	-1.21	*	.	F	0.90	3.16
Arg	290	A	A	.	.	.	.	.	2.36	-1.71	*	.	F	0.90	3.57
Ser	291	A	.	.	.	.	T	.	3.06	-1.71	*	.	F	1.30	6.92
Gln	292	A	.	.	.	.	T	.	2.24	-2.21	*	.	F	1.30	7.83
Arg	293	A	.	.	.	.	T	.	2.02	-1.53	.	.	F	1.30	3.30
Arg	294	A	.	.	.	.	T	.	1.17	-0.84	.	.	F	1.30	2.03
Arg	295	.	.	.	B	T	.	.	0.84	-0.59	.	*	F	1.15	0.87
Leu	296	.	.	B	B	.	.	.	0.56	-0.56	.	*	.	0.60	0.69
Leu	297	.	.	B	B	.	.	.	0.56	-0.06	.	*	.	0.30	0.35
Val	298	.	.	.	B	.	.	C	0.44	0.34	*	*	.	0.20	0.29
Pro	299	.	.	.	.	.	T	C	-0.01	0.34	*	.	.	0.90	0.61
Ala	300	.	.	.	.	.	T	C	-0.12	0.09	*	*	F	1.35	0.73

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Asn	301	.	.	.	.	.	T	C	0.48	-0.60	.	.	F	2.70	1.65
Glu	302	.	.	.	.	.	T	C	0.98	-0.81	.	.	F	3.00	1.65
Gly	303	.	.	.	.	.	.	C	1.83	-0.76	.	.	F	2.50	2.35
Asp	304	.	.	.	.	.	T	C	1.73	-1.26	.	.	F	2.40	2.54
Pro	305	.	.	.	.	.	T	C	1.51	-1.17	.	*	F	2.10	2.11
Thr	306	A	.	.	.	.	T	.	1.62	-0.49	.	*	F	1.30	1.76
Glu	307	A	.	.	.	.	T	.	1.62	-0.91	*	*	F	1.30	2.07
Thr	308	A	.	.	B	.	.	.	1.30	-0.51	*	*	F	0.90	2.31
Leu	309	A	.	.	B	.	.	.	0.60	-0.37	*	*	F	0.45	0.86
Arg	310	A	.	.	B	.	.	.	0.81	-0.07	*	*	.	0.30	0.43
Gln	311	A	.	.	B	.	.	.	1.12	-0.07	*	*	.	0.30	0.50
Cys	312	A	.	.	.	.	T	.	0.42	-0.56	*	*	.	1.15	1.01
Phe	313	A	.	.	.	.	T	.	0.14	-0.46	*	*	.	0.70	0.45
Asp	314	.	.	.	.	T	T	.	0.96	0.04	*	*	.	0.50	0.26
Asp	315	A	.	.	.	.	T	.	0.03	-0.36	*	*	.	0.70	0.81
Phe	316	A	A	.	.	.	.	.	-0.82	-0.24	*	.	.	0.30	0.77
Ala	317	A	A	.	.	.	.	.	-0.37	-0.39	*	.	.	0.30	0.34
Asp	318	A	A	.	.	.	.	.	-0.37	0.04	*	*	.	-0.30	0.32
Leu	319	A	A	.	.	.	.	.	-0.37	0.83	.	.	.	-0.60	0.32
Val	320	.	A	.	.	.	.	C	-0.67	0.04	.	.	.	-0.10	0.52
Pro	321	.	A	.	.	.	.	C	-0.26	-0.07	.	.	.	0.50	0.42
Phe	322	.	.	.	.	T	T	.	0.33	0.84	.	.	.	0.20	0.54
Asp	323	A	.	.	.	.	T	.	0.12	0.16	.	.	.	0.25	1.25
Ser	324	A	.	.	.	.	T	.	0.12	-0.06	.	.	F	1.00	1.25
Trp	325	A	.	.	.	.	T	.	0.38	0.20	*	*	F	0.40	1.19
Glu	326	A	A	.	.	.	.	.	0.70	0.03	*	.	F	-0.15	0.71
Pro	327	A	A	.	.	.	.	.	1.44	0.03	*	.	.	-0.15	1.03
Leu	328	A	A	.	.	.	.	.	0.63	-0.36	*	.	.	0.45	1.96
Met	329	A	A	.	.	.	.	.	0.59	-0.59	*	.	.	0.60	0.93
Arg	330	A	A	.	.	.	.	.	0.07	-0.16	*	.	.	0.30	0.60
Lys	331	A	A	.	.	.	.	.	-0.53	0.10	*	.	.	-0.30	0.60
Leu	332	A	A	.	.	.	.	.	-0.32	0.03	*	.	.	-0.30	0.60
Gly	333	A	A	.	.	.	.	.	0.49	-0.59	*	.	.	0.60	0.51
Leu	334	A	A	.	.	.	.	.	1.09	-0.19	*	.	.	0.30	0.41
Met	335	A	A	.	.	.	.	.	0.09	-0.19	*	*	.	0.30	0.86
Asp	336	A	A	.	.	.	.	.	0.09	-0.19	.	*	F	0.45	0.61
Asn	337	A	A	.	.	.	.	.	0.04	-0.61	*	*	F	0.90	1.48
Glu	338	A	A	.	.	.	.	.	-0.20	-0.66	*	*	F	0.90	1.11
Ile	339	A	A	.	.	.	.	.	0.66	-0.77	*	*	F	0.75	0.67
Lys	340	A	A	.	.	.	.	.	0.67	-0.77	.	*	F	0.75	0.83
Val	341	A	A	.	.	.	.	.	0.67	-0.67	.	*	.	0.60	0.49
Ala	342	A	A	.	.	.	.	.	0.08	-0.67	.	.	.	0.75	1.20
Lys	343	A	A	.	.	.	.	.	-0.51	-0.86	.	*	.	0.60	0.61
Ala	344	A	A	.	.	.	.	.	0.03	-0.36	.	*	.	0.30	0.83
Glu	345	A	A	.	.	.	.	.	-0.04	-0.57	*	.	.	0.60	0.81
Ala	346	A	A	.	.	.	.	.	0.92	-0.57	*	.	.	0.60	0.55
Ala	347	A	A	.	.	.	.	.	1.51	-0.57	.	*	.	0.75	1.07
Gly	348	A	.	.	.	.	.	.	1.16	-1.07	.	*	.	0.95	1.03
His	349	A	.	.	.	.	T	.	0.93	-0.59	.	.	.	1.15	1.47
Arg	350	A	.	.	.	.	T	.	0.69	-0.40	.	.	F	1.00	1.20

Table 4 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Asp	351	A	.	.	.	.	T	.	0.97	-0.14	.	.	F	1.00	1.90
Thr	352	A	.	.	.	.	T	.	0.96	-0.09	.	.	F	1.00	2.02
Leu	353	A	.	.	B	.	.	.	0.49	0.03	.	.	.	-0.15	1.02
Tyr	354	A	.	.	B	.	.	.	-0.37	0.71	.	*	.	-0.60	0.50
Thr	355	A	.	.	B	.	.	.	-0.43	1.40	.	*	.	-0.60	0.24
Met	356	A	.	.	B	.	.	.	-0.72	0.91	*	.	.	-0.60	0.59
Leu	357	A	.	.	B	.	.	.	-1.27	1.14	*	.	.	-0.60	0.40
Ile	358	A	.	.	B	.	.	.	-0.46	1.03	*	*	.	-0.60	0.20
Lys	359	A	.	.	B	.	.	.	-0.17	0.94	*	*	.	-0.60	0.33
Trp	360	A	.	.	B	.	.	.	-0.17	0.33	*	*	.	0.00	0.81
Val	361	A	.	.	B	.	.	.	0.09	0.13	*	*	.	0.45	1.66
Asn	362	.	.	.	.	.	T	C	1.01	-0.13	*	.	F	1.95	0.82
Lys	363	.	.	.	.	.	T	C	1.90	-0.13	*	*	F	2.40	1.53
Thr	364	.	.	.	.	.	T	C	1.27	-1.04	*	.	F	3.00	3.44
Gly	365	.	.	.	.	.	T	C	1.26	-1.19	*	.	F	2.70	2.16
Arg	366	.	A	.	.	T	.	.	1.26	-1.20	*	.	F	2.20	1.45
Asp	367	.	A	.	.	.	.	C	1.22	-0.56	*	.	F	1.55	0.75
Ala	368	A	A	.	.	.	.	.	0.87	-0.54	.	.	F	1.20	1.03
Ser	369	A	A	.	.	.	.	.	0.37	-0.49	.	.	.	0.30	0.76
Val	370	A	A	.	.	.	.	.	-0.10	0.20	.	*	.	-0.30	0.37
His	371	A	A	.	.	.	.	.	-0.21	0.89	.	*	.	-0.60	0.30
Thr	372	A	A	.	.	.	.	.	-0.80	0.39	*	*	.	-0.30	0.38
Leu	373	A	A	.	.	.	.	.	-1.02	0.50	*	*	.	-0.60	0.52
Leu	374	A	A	.	.	.	.	.	-0.72	0.54	*	.	.	-0.60	0.31
Asp	375	A	A	.	.	.	.	.	-0.18	0.04	*	.	.	-0.30	0.38
Ala	376	A	A	.	.	.	.	.	-0.96	0.04	*	.	.	-0.30	0.66
Leu	377	A	A	.	.	.	.	.	-0.99	0.04	*	.	.	-0.30	0.66
Glu	378	A	A	.	.	.	.	.	-0.18	-0.21	*	.	.	0.30	0.39
Thr	379	A	A	.	.	.	.	.	0.74	-0.21	*	*	F	0.45	0.67
Leu	380	A	A	.	.	.	.	.	-0.07	-0.71	*	.	F	0.90	1.59
Gly	381	A	A	.	.	.	.	.	-0.07	-0.71	*	.	F	0.75	0.76
Glu	382	A	A	.	.	.	.	.	0.79	-0.21	*	.	F	0.45	0.53
Arg	383	A	A	.	.	.	.	.	0.79	-0.70	*	.	F	0.90	1.28
Leu	384	A	A	.	.	.	.	.	1.14	-0.99	*	*	F	0.90	2.24
Ala	385	A	A	.	.	.	.	.	1.07	-1.41	*	*	F	0.90	2.59
Lys	386	A	A	.	.	.	.	.	1.41	-0.73	*	.	F	0.75	0.93
Gln	387	A	A	.	.	.	.	.	1.41	-0.73	*	*	F	0.90	1.95
Lys	388	A	A	.	.	.	.	.	1.27	-1.41	*	*	F	0.90	3.22
Ile	389	A	A	.	.	.	.	.	1.27	-1.41	.	*	F	0.90	2.19
Glu	390	A	A	.	.	.	.	.	1.04	-0.73	*	*	F	0.90	1.04
Asp	391	A	A	.	.	.	.	.	0.70	-0.44	.	*	F	0.45	0.43
His	392	A	A	.	.	.	.	.	0.40	-0.06	*	*	.	0.30	0.82
Leu	393	A	A	.	.	.	.	.	0.01	-0.36	*	*	.	0.30	0.64
Leu	394	A	A	.	.	.	.	.	0.94	0.07	*	*	F	-0.15	0.38
Ser	395	A	.	.	.	.	T	.	0.24	0.07	*	*	F	0.25	0.55
Ser	396	A	.	.	.	.	T	.	-0.36	0.36	*	*	F	0.25	0.58
Gly	397	.	.	.	.	T	T	.	-0.57	0.29	.	.	F	0.65	0.70
Lys	398	A	.	.	.	.	T	.	-0.57	0.36	.	.	F	0.25	0.82
Phe	399	A	A	.	.	.	.	.	0.24	0.66	.	.	.	-0.60	0.50
Met	400	.	A	B	.	.	.	.	0.20	0.27	.	*	.	-0.30	0.88

**Table 4 (continued)**

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Tyr	401	.	A	B	.	.	.	.	0.50	0.27	.	*	.	-0.30	0.44
Leu	402	A	A	.	.	.	.	.	0.26	0.67	.	*	.	-0.60	0.81
Glu	403	A	A	.	.	.	.	.	0.21	0.39	.	*	.	-0.30	0.82
Gly	404	A	.	.	.	.	.	.	0.61	-0.23	.	*	F	0.65	0.88
Asn	405	A	.	.	.	.	T	.	0.62	-0.60	.	*	F	1.30	1.43
Ala	406	A	.	.	.	.	T	.	0.27	-0.79	.	*	F	1.15	0.83
Asp	407	A	.	.	.	.	T	.	0.78	-0.17	.	*	F	0.85	0.83
Ser	408	A	.	.	.	.	T	.	0.39	-0.21	.	*	F	0.85	0.69
Ala	409	A	.	.	.	.	.	.	0.34	-0.19	.	*	.	0.50	0.88
Met	410	A	.	.	.	.	.	.	-0.04	-0.26	.	.	.	0.50	0.67
Ser	411	A	.	.	.	.	.	.	0.16	0.17	.	.	.	-0.10	0.64

[0143] In another aspect, the invention provides an antibody that binds a peptide or polypeptide comprising, or alternatively, consisting of, one, two, three, four, five or more, epitope-bearing portions of a TR7. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen et al., *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983).

[0144] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, J.G. Sutcliffe et al., "Antibodies That React With Predetermined Sites on Proteins," *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0145] Antigenic epitope-bearing peptides and polypeptides are therefore useful to raise antibodies, including monoclonal antibodies, that bind to a TR7 polypeptide. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of SEQ ID NO:3.

[0146] Antibodies of the invention may bind one or more antigenic TR7 polypeptides or peptides including, but not limited to: a polypeptide comprising, or alternatively consisting of, amino acid residues from about 62 to about 110 of SEQ ID NO:3, about 119 to about 164 of SEQ ID NO:3, about 224 to about 271 of SEQ ID NO:3, about 275 to about 370 of SEQ ID NO:3, about 69 to about 80 of SEQ ID NO:3, about 88 to about 95 of SEQ ID NO:3, about 99 to about 103 of SEQ ID NO:3, about 119 to about 123 of SEQ ID NO:3, about 130 to about 135 of SEQ ID NO:3, about 152 to about 163 of SEQ ID NO:3, about 226 to about 238 of SEQ ID NO:3, about 275 to about 279 of SEQ ID NO:3,



about 301 to about 305 of SEQ ID NO:3, and/or about 362 to about 367 of SEQ ID NO:3. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the TR7 receptor protein.

[0147] Epitope-bearing TR7 peptides and polypeptides may be produced by any conventional means. R.A. Houghten, "General Method for the Rapid Solid-Phase Synthesis of Large Numbers of Peptides: Specificity of Antigen-Antibody Interaction at the Level of Individual Amino Acids," *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986).

[0148] As one of skill in the art will appreciate, TR7 receptor polypeptides and the epitope-bearing fragments thereof described herein (e.g., corresponding to a portion of the extracellular domain, such as, for example, amino acid residues 52 to 184 of SEQ ID NO:3 can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric TR7 protein or protein fragment alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). TR7 fusion proteins may be used as an immunogen to elicit anti-TR7 antibodies. Thus, antibodies of the invention may bind fusion proteins that comprise all or a portion of a TR4 polypeptide such as TR7.

[0149] Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may also bind such modified TR7 polypeptides or TR7 polypeptide fragments or variants.

[0150] For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function or loss of the ability to be bound by a specific antibody. However, even if deletion of one or more amino acids from the N-terminus or C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other TR7 functional activities may still be retained. For example, in many instances, the ability of the shortened protein to induce and/or bind to antibodies which recognize TR7 (preferably antibodies that bind specifically to TR7) will be retained irrespective of the size or location of the deletion. In fact, polypeptides composed of as few as six TR7 amino acid residues may often evoke an immune response. Whether a particular polypeptide lacking N-terminal and/or C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0151] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind TR7 ligand) may still be retained. For example, the ability of shortened TR7 polypeptides to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a TR7 polypeptide with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities.

[0152] Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the amino terminus of the TR7 amino acid sequence shown in SEQ ID NO:3 up to the alanine residue at position number 406 and polynucleotides encoding such polypeptides. In particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues  $n^5$ -411 of SEQ ID NO:3 where  $n^5$  is an integer from 2 to 406 corresponding to the position of the amino acid residue in SEQ ID NO:3.

[0153] More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: E-2 to S-411; Q-3 to S-411; R-4 to S-411; G-5 to S-411; Q-6 to S-411; N-7 to S-411; A-8 to S-411; P-9 to S-411; A-10 to S-411; A-11 to S-411; S-12 to S-411; G-13 to S-411; A-14 to S-411; R-15 to S-411; K-16 to S-411; R-17 to S-411; H-18 to S-411; G-19 to S-411; P-20 to S-411; G-21 to S-411; P-22 to S-411; R-23 to S-411; E-24 to S-411; A-25 to S-411; R-26 to S-411; G-27 to S-411; A-28 to S-411; R-29 to S-411; P-30 to S-411; G-31 to S-411; P-32 to S-411; R-33 to S-411; V-34 to S-411; P-35 to S-411; K-36 to S-411; T-37 to S-411; L-38 to S-411; V-39 to S-411; L-40 to S-411; V-41 to S-411; V-42 to S-411; A-43 to S-411; A-44 to S-411; V-45 to S-411; L-46 to S-411; L-47 to S-411; L-48 to S-411; V-49 to S-411; S-50 to S-411; A-51 to S-411; E-52 to S-411; S-53 to S-411; A-54 to S-411; L-55 to S-411; I-56 to S-411; T-57 to S-411; Q-58 to S-411; Q-59 to S-411; D-60 to S-411; L-61 to S-411; A-62 to S-411; P-63 to S-411; Q-64 to S-411; Q-65 to S-411; R-66 to S-411; A-67 to S-411; A-68 to S-411; P-69 to S-411; Q-70 to S-411; Q-71 to S-411; K-72 to S-411; R-73 to S-411; S-74 to S-411; S-75 to S-411; P-76 to S-411; S-77 to S-411; E-78 to S-411; G-79 to S-411; L-80 to S-411; C-81 to S-411; P-82 to S-411; P-83 to S-411; G-84 to S-411; H-85 to S-411; H-86 to S-411; I-87 to S-411; S-88 to S-411; E-89 to S-411; D-90 to S-411; G-91 to S-411; R-92 to S-411; D-93 to S-411; C-94 to S-411; I-95 to S-411; S-96 to S-411; C-97 to S-411; K-98 to S-411; Y-99 to S-411; G-100 to S-411; Q-101 to S-411; D-102 to S-411; Y-103 to S-411; S-104 to S-411; T-105 to S-411; H-106 to S-411; W-107 to S-411; N-108 to S-411; D-109 to S-411; L-110 to S-411; L-111 to S-411; F-112 to S-411; C-113 to S-411; L-114 to S-411; R-115 to S-411; C-116 to S-411; T-117 to S-411; R-118 to S-411; C-119 to S-411; D-120 to S-411; S-121 to S-411; G-122 to S-411; E-123 to S-411; V-124 to S-411; E-125 to S-411; L-126 to S-411; S-127 to S-411; P-128 to S-411; C-129 to S-411; T-130 to S-411; T-131 to S-411; T-132 to S-411; R-133 to S-411; N-134 to S-411; T-135 to S-411; V-136 to S-411; C-137 to S-411; Q-138 to S-411; C-139 to S-411; E-140 to S-411; E-141 to S-411; G-142 to S-411; T-143 to S-411; F-144 to S-411; R-145 to S-411; E-146 to S-411; E-147 to S-411; D-148 to S-411; S-149 to S-411; P-150 to S-411; E-151 to S-411; M-152 to S-411; C-153 to S-411; R-154 to S-411; K-155 to S-411; C-156 to S-411; R-157 to S-411; T-158 to S-411; G-159 to S-411; C-160 to S-411; P-161 to S-411; R-162 to S-411; G-163 to S-411; M-164 to S-411; V-165 to S-411; K-166 to S-411; V-167 to S-411; G-168 to S-411; D-169 to S-411; C-170 to S-411; T-171 to S-411; P-172 to S-411; W-173 to S-411; S-174 to S-411; D-175 to S-411; I-176

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**[0154]** In another embodiment, N-terminal deletions of the TR7 polypeptide can be described by the general formula  $n^6$  to 184 where  $n^6$  is a number from 1 to 179 corresponding to the amino acid sequence identified in SEQ ID NO:3. In specific embodiments, antibodies of the invention bind N terminal deletions of the TR7 comprising, or alternatively consisting of, the amino acid sequence of residues: E-2 to G-184; Q-3 to G-184; R-4 to G-184; G-5 to G-184; Q-6 to G-184; N-7 to G-184; A-8 to G-184; P-9 to G-184; A-10 to G-184; A-11 to G-184; S-12 to G-184; G-13 to G-184; A-14 to G-184; R-15 to G-184; K-16 to G-184; R-17 to G-184; H-18 to G-184; G-19 to G-184; P-20 to G-184; G-21 to G-184; P-22 to G-184; R-23 to G-184; E-24 to G-184; A-25 to G-184; R-26 to G-184; G-27 to G-184; A-28 to G-184; R-29 to G-184; P-30 to G-184; G-31 to G-184; P-32 to G-184; R-33 to G-184; V-34 to G-184; P-35 to G-184; K-36 to G-184; T-37 to G-184; L-38 to G-184; V-39 to G-184; L-40 to G-184; V-41 to G-184; V-42 to G-184; A-43 to G-184; A-44 to G-184; V-45 to G-184; L-46 to G-184; L-47 to G-184; L-48 to G-184; V-49 to G-184; S-50 to G-184; A-51 to G-184; E-52 to G-184; S-53 to G-184; A-54 to G-184; L-55 to G-184; I-56 to G-184; T-57 to G-184; Q-58 to G-184; Q-59 to G-184; D-60 to G-184; L-61 to G-184; A-62 to G-184; P-63 to G-184; Q-64 to G-184; Q-65 to G-184; R-66 to G-184; A-67 to G-184; A-68 to G-184; P-69 to G-184; Q-70 to G-184; Q-71 to G-184; K-72 to G-184; R-73 to G-184; S-74 to G-184; S-75 to G-184; P-76 to G-184; S-77 to G-184; E-78 to G-184; G-79 to G-184; L-80 to G-184; C-81 to G-184; P-82 to G-184; P-83 to G-184; G-84 to G-184; H-85 to G-184; H-86 to G-184; I-87 to G-184; S-88 to G-184; E-89 to G-184; D-90 to G-184; G-91 to G-184; R-92 to G-184; D-93 to G-184; C-94 to G-184; I-95 to G-184; S-96 to G-184; C-97 to G-184; K-98 to G-184; Y-99

to G-184; G-100 to G-184; Q-101 to G-184; D-102 to G-184; Y-103 to G-184; S-104 to G-184; T-105 to G-184; H-106 to G-184; W-107 to G-184; N-108 to G-184; D-109 to G-184; L-110 to G-184; L-111 to G-184; F-112 to G-184; C-113 to G-184; L-114 to G-184; R-115 to G-184; C-116 to G-184; T-117 to G-184; R-118 to G-184; C-119 to G-184; D-120 to G-184; S-121 to G-184; G-122 to G-184; E-123 to G-184; V-124 to G-184; E-125 to G-184; L-126 to G-184; S-127 to G-184; P-128 to G-184; C-129 to G-184; T-130 to G-184; T-131 to G-184; T-132 to G-184; R-133 to G-184; N-134 to G-184; T-135 to G-184; V-136 to G-184; C-137 to G-184; Q-138 to G-184; C-139 to G-184; E-140 to G-184; E-141 to G-184; G-142 to G-184; T-143 to G-184; F-144 to G-184; R-145 to G-184; E-146 to G-184; E-147 to G-184; D-148 to G-184; S-149 to G-184; P-150 to G-184; E-151 to G-184; M-152 to G-184; C-153 to G-184; R-154 to G-184; K-155 to G-184; C-156 to G-184; R-157 to G-184; T-158 to G-184; G-159 to G-184; C-160 to G-184; P-161 to G-184; R-162 to G-184; G-163 to G-184; M-164 to G-184; V-165 to G-184; K-166 to G-184; V-167 to G-184; G-168 to G-184; D-169 to G-184; C-170 to G-184; T-171 to G-184; P-172 to G-184; W-173 to G-184; S-174 to G-184; D-175 to G-184; I-176 to G-184; E-177 to G-184; C-178 to G-184; and/or V-179 to G-184; of the TR7 extracellular domain sequence shown in SEQ ID NO:3.

**[0155]** Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind TR7 ligand (e.g., TRAIL)) may still be retained. For example, the ability of the shortened TR7 polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a TR7 polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six TR7 amino acid residues may often evoke an immune response.

**[0156]** Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the TR7 polypeptide shown in SEQ ID NO:3 up to the glutamic acid

residue at position number 52. In particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues 52-m<sup>5</sup> of SEQ ID NO:3, where m<sup>5</sup> is an integer from 57 to 410 corresponding to the position of the amino acid residue in SEQ ID NO:3.

**[0157]** More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues: E-52 to M-410; E-52 to A-409; E-52 to S-408; E-52 to D-407; E-52 to A-406; E-52 to N-405; E-52 to G-404; E-52 to E-403; E-52 to L-402; E-52 to Y-401; E-52 to M-400; E-52 to F-399; E-52 to K-398; E-52 to G-397; E-52 to S-396; E-52 to S-395; E-52 to L-394; E-52 to L-393; E-52 to H-392; E-52 to D-391; E-52 to E-390; E-52 to I-389; E-52 to K-388; E-52 to Q-387; E-52 to K-386; E-52 to A-385; E-52 to L-384; E-52 to R-383; E-52 to E-382; E-52 to G-381; E-52 to L-380; E-52 to T-379; E-52 to E-378; E-52 to L-377; E-52 to A-376; E-52 to D-375; E-52 to L-374; E-52 to L-373; E-52 to T-372; E-52 to H-371; E-52 to V-370; E-52 to S-369; E-52 to A-368; E-52 to D-367; E-52 to R-366; E-52 to G-365; E-52 to T-364; E-52 to K-363; E-52 to N-362; E-52 to V-361; E-52 to W-360; E-52 to K-359; E-52 to I-358; E-52 to L-357; E-52 to M-356; E-52 to T-355; E-52 to Y-354; E-52 to L-353; E-52 to T-352; E-52 to D-351; E-52 to R-350; E-52 to H-349; E-52 to G-348; E-52 to A-347; E-52 to A-346; E-52 to E-345; E-52 to A-344; E-52 to K-343; E-52 to A-342; E-52 to V-341; E-52 to K-340; E-52 to I-339; E-52 to E-338; E-52 to N-337; E-52 to D-336; E-52 to M-335; E-52 to L-334; E-52 to G-333; E-52 to L-332; E-52 to K-331; E-52 to R-330; E-52 to M-329; E-52 to L-328; E-52 to P-327; E-52 to E-326; E-52 to W-325; E-52 to S-324; E-52 to D-323; E-52 to F-322; E-52 to P-321; E-52 to V-320; E-52 to L-319; E-52 to D-318; E-52 to A-317; E-52 to F-316; E-52 to D-315; E-52 to D-314; E-52 to F-313; E-52 to C-312; E-52 to Q-311; E-52 to R-310; E-52 to L-309; E-52 to T-308; E-52 to E-307; E-52 to T-306; E-52 to P-305; E-52 to D-304; E-52 to G-303; E-52 to E-302; E-52 to N-301; E-52 to A-300; E-52 to P-299; E-52 to V-298; E-52 to L-297; E-52 to L-296; E-52 to R-295; E-52 to R-294; E-52 to R-293; E-52 to Q-292; E-52 to S-291; E-52 to R-290; E-52 to E-289; E-52 to A-288; E-52 to E-287; E-52 to A-286; E-52 to P-285; E-52 to E-284; E-52 to L-283; E-52 to L-282; E-52 to H-281; E-52 to E-280; E-52 to S-279; E-52 to E-278; E-52 to G-277; E-52 to P-276; E-52 to S-275; E-52 to L-274; E-52 to M-273; E-52 to N-272; E-52 to V-271; E-52 to G-270; E-52 to T-269; E-52 to P-268; E-52 to E-267; E-52 to A-266; E-52 to P-265; E-52 to E-264; E-52 to Q-263; E-52 to V-262; E-52 to E-261; E-52 to M-260; E-52 to E-259; E-52 to Q-258; E-52 to E-257; E-52 to P-256; E-52 to V-255; E-52

to Q-254; E-52 to T-253; E-52 to P-252; E-52 to Q-251; E-52 to L-250; E-52 to I-249; E-52 to S-248; E-52 to V-247; E-52 to I-246; E-52 to E-245; E-52 to N-244; E-52 to L-243; E-52 to V-242; E-52 to N-241; E-52 to D-240; E-52 to E-239; E-52 to A-238; E-52 to G-237; E-52 to P-236; E-52 to R-235; E-52 to Q-234; E-52 to S-233; E-52 to S-232; E-52 to R-231; E-52 to D-230; E-52 to V-229; E-52 to R-228; E-52 to E-227; E-52 to P-226; E-52 to D-225; E-52 to G-224; E-52 to G-223; E-52 to G-222; E-52 to G-221; E-52 to S-220; E-52 to C-219; E-52 to I-218; E-52 to G-217; E-52 to K-216; E-52 to L-215; E-52 to Y-214; E-52 to P-213; E-52 to L-212; E-52 to V-211; E-52 to K-210; E-52 to K-209; E-52 to W-208; E-52 to L-207; E-52 to L-206; E-52 to S-205; E-52 to K-204; E-52 to C-203; E-52 to V-202; E-52 to F-201; E-52 to V-200; E-52 to A-199; E-52 to V-198; E-52 to I-197; E-52 to L-196; E-52 to V-195; E-52 to V-194; E-52 to A-193; E-52 to A-192; E-52 to V-191; E-52 to T-190; E-52 to V-189; E-52 to G-188; E-52 to I-187; E-52 to I-186; E-52 to I-185; E-52 to G-184; E-52 to S-183; E-52 to E-182; E-52 to K-181; E-52 to H-180; E-52 to V-179; E-52 to C-178; E-52 to E-177; E-52 to I-176; E-52 to D-175; E-52 to S-174; E-52 to W-173; E-52 to P-172; E-52 to T-171; E-52 to C-170; E-52 to D-169; E-52 to G-168; E-52 to V-167; E-52 to K-166; E-52 to V-165; E-52 to M-164; E-52 to G-163; E-52 to R-162; E-52 to P-161; E-52 to C-160; E-52 to G-159; E-52 to T-158; E-52 to R-157; E-52 to C-156; E-52 to K-155; E-52 to R-154; E-52 to C-153; E-52 to M-152; E-52 to E-151; E-52 to P-150; E-52 to S-149; E-52 to D-148; E-52 to E-147; E-52 to E-146; E-52 to R-145; E-52 to F-144; E-52 to T-143; E-52 to G-142; E-52 to E-141; E-52 to E-140; E-52 to C-139; E-52 to Q-138; E-52 to C-137; E-52 to V-136; E-52 to T-135; E-52 to N-134; E-52 to R-133; E-52 to T-132; E-52 to T-131; E-52 to T-130; E-52 to C-129; E-52 to P-128; E-52 to S-127; E-52 to L-126; E-52 to E-125; E-52 to V-124; E-52 to E-123; E-52 to G-122; E-52 to S-121; E-52 to D-120; E-52 to C-119; E-52 to R-118; E-52 to T-117; E-52 to C-116; E-52 to R-115; E-52 to L-114; E-52 to C-113; E-52 to F-112; E-52 to L-111; E-52 to L-110; E-52 to D-109; E-52 to N-108; E-52 to W-107; E-52 to H-106; E-52 to T-105; E-52 to S-104; E-52 to Y-103; E-52 to D-102; E-52 to Q-101; E-52 to G-100; E-52 to Y-99; E-52 to K-98; E-52 to C-97; E-52 to S-96; E-52 to I-95; E-52 to C-94; E-52 to D-93; E-52 to R-92; E-52 to G-91; E-52 to D-90; E-52 to E-89; E-52 to S-88; E-52 to I-87; E-52 to H-86; E-52 to H-85; E-52 to G-84; E-52 to P-83; E-52 to P-82; E-52 to C-81; E-52 to L-80; E-52 to G-79; E-52 to E-78; E-52 to S-77; E-52 to P-76; E-52 to S-75; E-52 to S-74; E-52 to R-73; E-52 to K-72; E-52 to Q-71; E-52 to Q-70; E-52 to P-69; E-52 to A-68; E-52 to A-67; E-52 to R-66; E-52 to Q-65; E-52 to Q-64; E-52 to P-63; E-52 to A-62; E-52



to L-61; E-52 to D-60; E-52 to Q-59; E-52 to Q-58; and/or E-52 to T-57; of the TR7 sequence shown in SEQ ID NO:3.

**[0158]** In another embodiment, antibodies of the invention bind C-terminal deletions of the TR7 polypeptide that can be described by the general formula 52-m<sup>6</sup> where m<sup>6</sup> is a number from 57 to 183 corresponding to the amino acid sequence identified in SEQ ID NO:3. In specific embodiments, antibodies of the invention bind C terminal deletions of the TR7 polypeptide comprising, or alternatively, consisting of, amino acid residues: E-52 to S-183; E-52 to E-182; E-52 to K-181; E-52 to H-180; E-52 to V-179; E-52 to C-178; E-52 to E-177; E-52 to I-176; E-52 to D-175; E-52 to S-174; E-52 to W-173; E-52 to P-172; E-52 to T-171; E-52 to C-170; E-52 to D-169; E-52 to G-168; E-52 to V-167; E-52 to K-166; E-52 to V-165; E-52 to M-164; E-52 to G-163; E-52 to R-162; E-52 to P-161; E-52 to C-160; E-52 to G-159; E-52 to T-158; E-52 to R-157; E-52 to C-156; E-52 to K-155; E-52 to R-154; E-52 to C-153; E-52 to M-152; E-52 to E-151; E-52 to P-150; E-52 to S-149; E-52 to D-148; E-52 to E-147; E-52 to E-146; E-52 to R-145; E-52 to F-144; E-52 to T-143; E-52 to G-142; E-52 to E-141; E-52 to E-140; E-52 to C-139; E-52 to Q-138; E-52 to C-137; E-52 to V-136; E-52 to T-135; E-52 to N-134; E-52 to R-133; E-52 to T-132; E-52 to T-131; E-52 to T-130; E-52 to C-129; E-52 to P-128; E-52 to S-127; E-52 to L-126; E-52 to E-125; E-52 to V-124; E-52 to E-123; E-52 to G-122; E-52 to S-121; E-52 to D-120; E-52 to C-119; E-52 to R-118; E-52 to T-117; E-52 to C-116; E-52 to R-115; E-52 to L-114; E-52 to C-113; E-52 to F-112; E-52 to L-111; E-52 to L-110; E-52 to D-109; E-52 to N-108; E-52 to W-107; E-52 to H-106; E-52 to T-105; E-52 to S-104; E-52 to Y-103; E-52 to D-102; E-52 to Q-101; E-52 to G-100; E-52 to Y-99; E-52 to K-98; E-52 to C-97; E-52 to S-96; E-52 to I-95; E-52 to C-94; E-52 to D-93; E-52 to R-92; E-52 to G-91; E-52 to D-90; E-52 to E-89; E-52 to S-88; E-52 to I-87; E-52 to H-86; E-52 to H-85; E-52 to G-84; E-52 to P-83; E-52 to P-82; E-52 to C-81; E-52 to L-80; E-52 to G-79; E-52 to E-78; E-52 to S-77; E-52 to P-76; E-52 to S-75; E-52 to S-74; E-52 to R-73; E-52 to K-72; E-52 to Q-71; E-52 to Q-70; E-52 to P-69; E-52 to A-68; E-52 to A-67; E-52 to R-66; E-52 to Q-65; E-52 to Q-64; E-52 to P-63; E-52 to A-62; E-52 to L-61; E-52 to D-60; E-52 to Q-59; E-52 to Q-58; and/or E-52 to T-57; of the TR7 extracellular domain sequence shown in SEQ ID NO:3.

**[0159]** The invention also provides antibodies that bind polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a TR7

polypeptide, which may be described generally as having residues  $n^5$ - $m^5$  and/or  $n^6$ - $m^6$  of SEQ ID NO:3, where  $n^5$ ,  $n^6$ ,  $m^5$ , and  $m^6$  are integers as described above.

**[0160]** Also included are antibodies that bind a polypeptide consisting of a portion of the complete TR7 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97920, where this portion excludes from 1 to about 78 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97920, or from 1 to about 233 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97920.

**[0161]** Preferably, antibodies of the present invention bind the N- and C-terminal deletion mutants comprising only a portion of the extracellular domain; i.e., within residues 52-184 of SEQ ID NO:3, since any portion therein is expected to be soluble.

**[0162]** It will be recognized in the art that some amino acid sequence of TR7 can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

**[0163]** Thus, the invention further includes antibodies that bind variations of the TR7 protein which show substantial TR7 protein activity or which include regions of TR7, such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U. *et al.*, *Science* 247:1306-1310 (1990).

**[0164]** Thus, antibodies of the present invention may bind a fragment, derivative, or analog of the polypeptide of SEQ ID NO:3, or that encoded by the cDNA in ATCC deposit 97920. Such fragments, variants or derivatives may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the

mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

**[0165]** Of particular interest are substitutions of charged amino acids with another charged amino acids and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TR7 protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al.* *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

**[0166]** The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al.*, *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the antibodies of the present invention may bind a TR7 receptor that contains one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

**[0167]** As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 3 above).

**[0168]** In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of SEQ ID NO:3 and/or any of the polypeptide fragments described herein (e.g., the extracellular domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2.

**[0169]** In specific embodiments, the antibodies of the invention bind TR7 polypeptides or fragments or variants thereof (especially a fragment comprising or alternatively consisting of, the extracellular soluble domain of TR7), that contains any one or more of the following conservative mutations in TR7: M1 replaced with A, G, I, L, S, T, or V; E2 replaced with D; Q3 replaced with N; R4 replaced with H, or K; G5 replaced

with A, I, L, S, T, M, or V; Q6 replaced with N; N7 replaced with Q; A8 replaced with G, I, L, S, T, M, or V; A10 replaced with G, I, L, S, T, M, or V; A11 replaced with G, I, L, S, T, M, or V; S12 replaced with A, G, I, L, T, M, or V; G13 replaced with A, I, L, S, T, M, or V; A14 replaced with G, I, L, S, T, M, or V; R15 replaced with H, or K; K16 replaced with H, or R; R17 replaced with H, or K; H18 replaced with K, or R; G19 replaced with A, I, L, S, T, M, or V; G21 replaced with A, I, L, S, T, M, or V; R23 replaced with H, or K; E24 replaced with D; A25 replaced with G, I, L, S, T, M, or V; R26 replaced with H, or K; G27 replaced with A, I, L, S, T, M, or V; A28 replaced with G, I, L, S, T, M, or V; R29 replaced with H, or K; G31 replaced with A, I, L, S, T, M, or V; R33 replaced with H, or K; V34 replaced with A, G, I, L, S, T, or M; K36 replaced with H, or R; T37 replaced with A, G, I, L, S, M, or V; L38 replaced with A, G, I, S, T, M, or V; V39 replaced with A, G, I, L, S, T, or M; L40 replaced with A, G, I, S, T, M, or V; V41 replaced with A, G, I, L, S, T, or M; V42 replaced with A, G, I, L, S, T, or M; A43 replaced with G, I, L, S, T, M, or V; A44 replaced with G, I, L, S, T, M, or V; V45 replaced with A, G, I, L, S, T, or M; L46 replaced with A, G, I, S, T, M, or V; L47 replaced with A, G, I, S, T, M, or V; L48 replaced with A, G, I, S, T, M, or V; V49 replaced with A, G, I, L, S, T, or M; S50 replaced with A, G, I, L, T, M, or V; A51 replaced with G, I, L, S, T, M, or V; E52 replaced with D; S53 replaced with A, G, I, L, T, M, or V; A54 replaced with G, I, L, S, T, M, or V; L55 replaced with A, G, I, S, T, M, or V; I56 replaced with A, G, L, S, T, M, or V; T57 replaced with A, G, I, L, S, M, or V; Q58 replaced with N; Q59 replaced with N; D60 replaced with E; L61 replaced with A, G, I, S, T, M, or V; A62 replaced with G, I, L, S, T, M, or V; Q64 replaced with N; Q65 replaced with N; R66 replaced with H, or K; A67 replaced with G, I, L, S, T, M, or V; A68 replaced with G, I, L, S, T, M, or V; Q70 replaced with N; Q71 replaced with N; K72 replaced with H, or R; R73 replaced with H, or K; S74 replaced with A, G, I, L, T, M, or V; S75 replaced with A, G, I, L, T, M, or V; S77 replaced with A, G, I, L, T, M, or V; E78 replaced with D; G79 replaced with A, I, L, S, T, M, or V; L80 replaced with A, G, I, S, T, M, or V; G84 replaced with A, I, L, S, T, M, or V; H85 replaced with K, or R; H86 replaced with K, or R; I87 replaced with A, G, L, S, T, M, or V; S88 replaced with A, G, I, L, T, M, or V; E89 replaced with D; D90 replaced with E; G91 replaced with A, I, L, S, T, M, or V; R92 replaced with H, or K; D93 replaced with E; I95 replaced with A, G, L, S, T, M, or V; S96 replaced with A, G, I, L, T, M, or V; K98 replaced with H, or R; Y99 replaced with F, or W; G100 replaced with A, I, L, S, T, M, or V; Q101 replaced with N; D102 replaced with E; Y103 replaced with F, or

W; S104 replaced with A, G, I, L, T, M, or V; T105 replaced with A, G, I, L, S, M, or V; H106 replaced with K, or R; W107 replaced with F, or Y; N108 replaced with Q; D109 replaced with E; L110 replaced with A, G, I, S, T, M, or V; L111 replaced with A, G, I, S, T, M, or V; F112 replaced with W, or Y; L114 replaced with A, G, I, S, T, M, or V; R115 replaced with H, or K; T117 replaced with A, G, I, L, S, M, or V; R118 replaced with H, or K; D120 replaced with E; S121 replaced with A, G, I, L, T, M, or V; G122 replaced with A, I, L, S, T, M, or V; E123 replaced with D; V124 replaced with A, G, I, L, S, T, or M; E125 replaced with D; L126 replaced with A, G, I, S, T, M, or V; S127 replaced with A, G, I, L, T, M, or V; T130 replaced with A, G, I, L, S, M, or V; T131 replaced with A, G, I, L, S, M, or V; T132 replaced with A, G, I, L, S, M, or V; R133 replaced with H, or K; N134 replaced with Q; T135 replaced with A, G, I, L, S, M, or V; V136 replaced with A, G, I, L, S, T, or M; Q138 replaced with N; E140 replaced with D; E141 replaced with D; G142 replaced with A, I, L, S, T, M, or V; T143 replaced with A, G, I, L, S, M, or V; F144 replaced with W, or Y; R145 replaced with H, or K; E146 replaced with D; E147 replaced with D; D148 replaced with E; S149 replaced with A, G, I, L, T, M, or V; E151 replaced with D; M152 replaced with A, G, I, L, S, T, or V; R154 replaced with H, or K; K155 replaced with H, or R; R157 replaced with H, or K; T158 replaced with A, G, I, L, S, M, or V; G159 replaced with A, I, L, S, T, M, or V; R162 replaced with H, or K; G163 replaced with A, I, L, S, T, M, or V; M164 replaced with A, G, I, L, S, T, or V; V165 replaced with A, G, I, L, S, T, or M; K166 replaced with H, or R; V167 replaced with A, G, I, L, S, T, or M; G168 replaced with A, I, L, S, T, M, or V; D169 replaced with E; T171 replaced with A, G, I, L, S, M, or V; W173 replaced with F, or Y; S174 replaced with A, G, I, L, T, M, or V; D175 replaced with E; I176 replaced with A, G, L, S, T, M, or V; E177 replaced with D; V179 replaced with A, G, I, L, S, T, or M; H180 replaced with K, or R; K181 replaced with H, or R; E182 replaced with D; S183 replaced with A, G, I, L, T, M, or V; G184 replaced with A, I, L, S, T, M, or V; I185 replaced with A, G, L, S, T, M, or V; I186 replaced with A, G, L, S, T, M, or V; I187 replaced with A, G, L, S, T, M, or V; G188 replaced with A, I, L, S, T, M, or V; V189 replaced with A, G, I, L, S, T, or M; T190 replaced with A, G, I, L, S, M, or V; V191 replaced with A, G, I, L, S, T, or M; A192 replaced with G, I, L, S, T, M, or V; A193 replaced with G, I, L, S, T, M, or V; V194 replaced with A, G, I, L, S, T, or M; V195 replaced with A, G, I, L, S, T, or M; L196 replaced with A, G, I, S, T, M, or V; I197 replaced with A, G, L, S, T, M, or V; V198 replaced with A, G, I, L, S, T, or M; A199 replaced with G, I, L, S, T, M, or V;

V200 replaced with A, G, I, L, S, T, or M; F201 replaced with W, or Y; V202 replaced with A, G, I, L, S, T, or M; K204 replaced with H, or R; S205 replaced with A, G, I, L, T, M, or V; L206 replaced with A, G, I, S, T, M, or V; L207 replaced with A, G, I, S, T, M, or V; W208 replaced with F, or Y; K209 replaced with H, or R; K210 replaced with H, or R; V211 replaced with A, G, I, L, S, T, or M; L212 replaced with A, G, I, S, T, M, or V; Y214 replaced with F, or W; L215 replaced with A, G, I, S, T, M, or V; K216 replaced with H, or R; G217 replaced with A, I, L, S, T, M, or V; I218 replaced with A, G, L, S, T, M, or V; S220 replaced with A, G, I, L, T, M, or V; G221 replaced with A, I, L, S, T, M, or V; G222 replaced with A, I, L, S, T, M, or V; G223 replaced with A, I, L, S, T, M, or V; G224 replaced with A, I, L, S, T, M, or V; D225 replaced with E; E227 replaced with D; R228 replaced with H, or K; V229 replaced with A, G, I, L, S, T, or M; D230 replaced with E; R231 replaced with H, or K; S232 replaced with A, G, I, L, T, M, or V; S233 replaced with A, G, I, L, T, M, or V; Q234 replaced with N; R235 replaced with H, or K; G237 replaced with A, I, L, S, T, M, or V; A238 replaced with G, I, L, S, T, M, or V; E239 replaced with D; D240 replaced with E; N241 replaced with Q; V242 replaced with A, G, I, L, S, T, or M; L243 replaced with A, G, I, S, T, M, or V; N244 replaced with Q; E245 replaced with D; I246 replaced with A, G, L, S, T, M, or V; V247 replaced with A, G, I, L, S, T, or M; S248 replaced with A, G, I, L, T, M, or V; I249 replaced with A, G, L, S, T, M, or V; L250 replaced with A, G, I, S, T, M, or V; Q251 replaced with N; T253 replaced with A, G, I, L, S, M, or V; Q254 replaced with N; V255 replaced with A, G, I, L, S, T, or M; E257 replaced with D; Q258 replaced with N; E259 replaced with D; M260 replaced with A, G, I, L, S, T, or V; E261 replaced with D; V262 replaced with A, G, I, L, S, T, or M; Q263 replaced with N; E264 replaced with D; A266 replaced with G, I, L, S, T, M, or V; E267 replaced with D; T269 replaced with A, G, I, L, S, M, or V; G270 replaced with A, I, L, S, T, M, or V; V271 replaced with A, G, I, L, S, T, or M; N272 replaced with Q; M273 replaced with A, G, I, L, S, T, or V; L274 replaced with A, G, I, S, T, M, or V; S275 replaced with A, G, I, L, T, M, or V; G277 replaced with A, I, L, S, T, M, or V; E278 replaced with D; S279 replaced with A, G, I, L, T, M, or V; E280 replaced with D; H281 replaced with K, or R; L282 replaced with A, G, I, S, T, M, or V; L283 replaced with A, G, I, S, T, M, or V; E284 replaced with D; A286 replaced with G, I, L, S, T, M, or V; E287 replaced with D; A288 replaced with G, I, L, S, T, M, or V; E289 replaced with D; R290 replaced with H, or K; S291 replaced with A, G, I, L, T, M, or V; Q292 replaced with N; R293 replaced with H, or K; R294 replaced with H, or K; R295

replaced with H, or K; L296 replaced with A, G, I, S, T, M, or V; L297 replaced with A, G, I, S, T, M, or V; V298 replaced with A, G, I, L, S, T, or M; A300 replaced with G, I, L, S, T, M, or V; N301 replaced with Q; E302 replaced with D; G303 replaced with A, I, L, S, T, M, or V; D304 replaced with E; T306 replaced with A, G, I, L, S, M, or V; E307 replaced with D; T308 replaced with A, G, I, L, S, M, or V; L309 replaced with A, G, I, S, T, M, or V; R310 replaced with H, or K; Q311 replaced with N; F313 replaced with W, or Y; D314 replaced with E; D315 replaced with E; F316 replaced with W, or Y; A317 replaced with G, I, L, S, T, M, or V; D318 replaced with E; L319 replaced with A, G, I, S, T, M, or V; V320 replaced with A, G, I, L, S, T, or M; F322 replaced with W, or Y; D323 replaced with E; S324 replaced with A, G, I, L, T, M, or V; W325 replaced with F, or Y; E326 replaced with D; L328 replaced with A, G, I, S, T, M, or V; M329 replaced with A, G, I, L, S, T, or V; R330 replaced with H, or K; K331 replaced with H, or R; L332 replaced with A, G, I, S, T, M, or V; G333 replaced with A, I, L, S, T, M, or V; L334 replaced with A, G, I, S, T, M, or V; M335 replaced with A, G, I, L, S, T, or V; D336 replaced with E; N337 replaced with Q; E338 replaced with D; I339 replaced with A, G, L, S, T, M, or V; K340 replaced with H, or R; V341 replaced with A, G, I, L, S, T, or M; A342 replaced with G, I, L, S, T, M, or V; K343 replaced with H, or R; A344 replaced with G, I, L, S, T, M, or V; E345 replaced with D; A346 replaced with G, I, L, S, T, M, or V; A347 replaced with G, I, L, S, T, M, or V; G348 replaced with A, I, L, S, T, M, or V; H349 replaced with K, or R; R350 replaced with H, or K; D351 replaced with E; T352 replaced with A, G, I, L, S, M, or V; L353 replaced with A, G, I, S, T, M, or V; Y354 replaced with F, or W; T355 replaced with A, G, I, L, S, M, or V; M356 replaced with A, G, I, L, S, T, or V; L357 replaced with A, G, I, S, T, M, or V; I358 replaced with A, G, L, S, T, M, or V; K359 replaced with H, or R; W360 replaced with F, or Y; V361 replaced with A, G, I, L, S, T, or M; N362 replaced with Q; K363 replaced with H, or R; T364 replaced with A, G, I, L, S, M, or V; G365 replaced with A, I, L, S, T, M, or V; R366 replaced with H, or K; D367 replaced with E; A368 replaced with G, I, L, S, T, M, or V; S369 replaced with A, G, I, L, T, M, or V; V370 replaced with A, G, I, L, S, T, or M; H371 replaced with K, or R; T372 replaced with A, G, I, L, S, M, or V; L373 replaced with A, G, I, S, T, M, or V; L374 replaced with A, G, I, S, T, M, or V; D375 replaced with E; A376 replaced with G, I, L, S, T, M, or V; L377 replaced with A, G, I, S, T, M, or V; E378 replaced with D; T379 replaced with A, G, I, L, S, M, or V; L380 replaced with A, G, I, S, T, M, or V; G381 replaced with A, I, L, S, T, M, or V; E382 replaced with D;

R383 replaced with H, or K; L384 replaced with A, G, I, S, T, M, or V; A385 replaced with G, I, L, S, T, M, or V; K386 replaced with H, or R; Q387 replaced with N; K388 replaced with H, or R; I389 replaced with A, G, L, S, T, M, or V; E390 replaced with D; D391 replaced with E; H392 replaced with K, or R; L393 replaced with A, G, I, S, T, M, or V; L394 replaced with A, G, I, S, T, M, or V; S395 replaced with A, G, I, L, T, M, or V; S396 replaced with A, G, I, L, T, M, or V; G397 replaced with A, I, L, S, T, M, or V; K398 replaced with H, or R; F399 replaced with W, or Y; M400 replaced with A, G, I, L, S, T, or V; Y401 replaced with F, or W; L402 replaced with A, G, I, S, T, M, or V; E403 replaced with D; G404 replaced with A, I, L, S, T, M, or V; N405 replaced with Q; A406 replaced with G, I, L, S, T, M, or V; D407 replaced with E; S408 replaced with A, G, I, L, T, M, or V; A409 replaced with G, I, L, S, T, M, or V; M410 replaced with A, G, I, L, S, T, or V; and/or S411 replaced with A, G, I, L, T, M, or V of SEQ ID NO:3.

**[0170]** In specific embodiments, the antibodies of the invention bind TR7 polypeptides or fragments or variants thereof (especially a fragment comprising or alternatively consisting of, the extracellular soluble domain of TR7), that contains any one or more of the following non-conservative mutations in TR7: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q3 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R4 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q6 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N7 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A8 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A11 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R15 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K16 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R17 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H18 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G19 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G21 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P22 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R23 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E24 replaced with H, K, R, A, G,



I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R26 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G27 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R29 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P30 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P32 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R33 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V34 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P35 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K36 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T37 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L40 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A43 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A44 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L46 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E52 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q58 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D60 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A62 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P63 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q64 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q65 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R66 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A68 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P69 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q70 replaced

with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q71 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K72 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R73 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S74 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P76 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E78 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L80 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C81 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P82 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P83 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G84 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H85 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H86 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I87 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S88 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E89 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D90 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R92 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D93 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C94 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C97 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K98 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y99 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q101 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D102 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y103 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S104 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H106 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W107 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N108 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D109 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F112 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; C113 replaced with D, E, H, K,

R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R115 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C116 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T117 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R118 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C119 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; D120 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G122 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E123 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V124 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E125 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S127 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P128 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C129 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T130 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T132 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R133 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N134 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V136 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C137 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q138 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C139 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; E140 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E141 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F144 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R145 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E146 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E147 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D148 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P150 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E151 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C153 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R154 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K155 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C;

C156 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R157 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T158 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C160 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P161 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R162 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M164 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V165 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K166 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G168 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D169 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; T171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P172 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W173 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D175 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I176 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E177 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C178 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; V179 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H180 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K181 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E182 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S183 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G184 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I186 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I187 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G188 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V189 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V191 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A192 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A193 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V194 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L196 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I197 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F201 replaced with D, E, H, K, R, N,

Q, A, G, I, L, S, T, M, V, P, or C; V202 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C203 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K204 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S205 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L206 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W208 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K209 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K210 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y214 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K216 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I218 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C219 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G222 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G223 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G224 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D225 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P226 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E227 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R228 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D230 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S232 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q234 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R235 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P236 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A238 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E239 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D240 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N241 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N244 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E245 replaced with H, K, R, A, G, I,

L, S, T, M, V, N, Q, F, W, Y, P, or C; I246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V247 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S248 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q251 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P252 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q254 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P256 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E257 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q258 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E259 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M260 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E261 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V262 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q263 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E264 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P265 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E267 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T269 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N272 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M273 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L274 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P276 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G277 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E278 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S279 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E280 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H281 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L282 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L283 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E284 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P285 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E287 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E289 replaced with

H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R290 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S291 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q292 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R293 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R294 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R295 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L296 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L297 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V298 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P299 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N301 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E302 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G303 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D304 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P305 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T306 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E307 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T308 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L309 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R310 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q311 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C312 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; F313 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D314 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D315 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F316 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D318 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L319 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V320 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P321 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F322 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D323 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S324 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W325 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E326 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P327 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L328 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M329 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R330 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K331 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y,

P, or C; L332 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G333 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L334 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M335 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D336 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N337 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E338 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I339 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K340 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V341 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A342 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K343 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A344 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E345 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A346 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A347 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H349 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R350 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D351 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T352 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L353 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y354 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T355 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M356 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L357 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I358 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K359 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W360 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V361 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N362 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K363 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T364 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G365 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R366 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D367 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A368 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S369 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V370 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H371 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T372 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L373 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L374 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D375 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A376 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L377 replaced



with D, E, H, K, R, N, Q, F, W, Y, P, or C; E378 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T379 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L380 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G381 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E382 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R383 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L384 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A385 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K386 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q387 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K388 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I389 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E390 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D391 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H392 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L393 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L394 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S395 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S396 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G397 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K398 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F399 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; M400 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y401 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L402 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E403 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G404 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N405 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A406 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D407 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S408 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A409 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M410 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or S411 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C of SEQ ID NO:3.

**[0171]** Amino acids in the TR7 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as

crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)). In preferred embodiments, antibodies of the present invention bind regions of TR7 that are essential for TR7 function. In other preferred embodiments, antibodies of the present invention bind regions of TR7 that are essential for TR7 function and inhibit or abolish TR7 function. In other preferred embodiments, antibodies of the present invention bind regions of TR7 that are essential for TR7 function and enhance TR7 function.

[0172] Additionally, protein engineering may be employed to improve or alter the characteristics of TR7 polypeptides. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or polypeptides including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may bind such modified TR7 polypeptides.

[0173] Non-naturally occurring TR7 variants that may be bound by the antibodies of the invention may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., Carter *et al.*, *Nucl. Acids Res.* 13:4331 (1986); and Zoller *et al.*, *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see e.g., Wells *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (see e.g., Wells *et al.*, *Philos. Trans. R. Soc. London SerA* 317:415 (1986)).

[0174] Thus, the invention also encompasses antibodies that bind TR7 derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to generate TR7 polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the TR7 polypeptides, and/or an amino acid deletion at the

second position of any one or more such recognition sequences will prevent glycosylation of the TR7 at the modified tripeptide sequence (see, e.g., Miyajimo *et al.*, *EMBO J* 5(6):1193-1197). Additionally, one or more of the amino acid residues of TR7 polypeptides (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins.

**[0175]** The antibodies of the present invention also include antibodies that bind a polypeptide comprising, or alternatively, consisting of the polypeptide encoded by the deposited cDNA (the deposit having ATCC Accession Number 97920) including the leader; the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); a polypeptide comprising or alternatively, consisting of, amino acids about 1 to about 411 in SEQ ID NO:3; a polypeptide comprising or alternatively, consisting of, amino acids about 2 to about 411 in SEQ ID NO:3; a polypeptide comprising or alternatively, consisting of, amino acids about 52 to about 411 in SEQ ID NO:3; a polypeptide comprising or alternatively, consisting of, the TR7 extracellular domain; a polypeptide comprising or alternatively, consisting of, the TR7 cysteine rich domain; a polypeptide comprising or alternatively, consisting of, the TR7 transmembrane domain; a polypeptide comprising or alternatively, consisting of, the TR7 intracellular domain; a polypeptide comprising or alternatively, consisting of, the extracellular and intracellular domains with all or part of the transmembrane domain deleted; and a polypeptide comprising or alternatively, consisting of, the TR7 death domain; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

**[0176]** By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TR7 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the TR7 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of

the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

**[0177]** As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in FIGs. 1A-B (SEQ ID NO:3), the amino acid sequence encoded by deposited cDNA clones, or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

**[0178]** In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the

query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

**[0179]** The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns and as a source for generating antibodies that bind the TR7 polypeptides, using methods well known to those of skill in the art.

**[0180]** The present application is also directed to antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to the TR7 polypeptide sequence set forth herein as n<sup>5</sup>-m<sup>5</sup>, and/or n<sup>6</sup>-m<sup>6</sup>. In preferred embodiments, the application is directed to antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific TR7 N- and C-terminal deletions recited herein.

[0181] In certain preferred embodiments, antibodies of the invention bind TR7 proteins of the invention comprise fusion proteins as described above wherein the TR7 polypeptides are those described as n<sup>5</sup>-m<sup>5</sup>, and n<sup>6</sup>-m<sup>6</sup>, herein.

***Antibodies of the invention may bind Modified TRAIL Receptor Polypeptides***

[0182] It is specifically contemplated that antibodies of the present invention may bind modified forms of TR4 proteins SEQ ID NO:1). In those embodiments where an antibody of the present invention specifically binds both TR4 and TR7 (SEQ ID NO:3), it is also specifically contemplated that those antibodies may bind modified forms of TR4 and/or TR7. Modified forms of TR7 would include, for example, modified forms of TR7 that correspond to the modified forms of TR4 described below.

[0183] In specific embodiments, antibodies of the present invention bind TR4 polypeptides (such as those described above) including, but not limited to naturally purified TR4 polypeptides, TR4 polypeptides produced by chemical synthetic procedures, and TR4 polypeptides produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells using, for example, the recombinant compositions and methods described above. Depending upon the host employed in a recombinant production procedure, the polypeptides may be glycosylated or non-glycosylated. In addition, TR4 polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0184] In addition, TR4 proteins that are bound by antibodies of the present invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y. (1983), and Hunkapiller, *et al.*, *Nature* 310:105-111 (1984)). For example, a peptide corresponding to a fragment of a TR4 polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the TR4 polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine,

cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

**[0185]** The invention additionally, encompasses antibodies that bind TR4 polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin; etc.

**[0186]** Additional post-translational modifications to TR4 polypeptides for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

**[0187]** Also provided by the invention are antibodies that bind chemically modified derivatives of TR4 polypeptides which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

**[0188]** The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on

biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0189] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0190] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, *e.g.*, EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0191] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (*e.g.*, lysine, histidine; aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue



(e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0192] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (*i.e.*, separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0193] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0194] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0195] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for

connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

**[0196]** The number of polyethylene glycol moieties attached to each TR4 polypeptide (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

**[0197]** As mentioned the antibodies of the present invention may bind TR4 polypeptides that are modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given TR4 polypeptide. TR4 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic TR4 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization,

selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol* 182:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci* 663:48-62 (1992)).

### **Anti-TR4 Antibodies**

[0198] In one embodiment, the invention provides antibodies (e.g., antibodies comprising two heavy chains and two light chains linked together by disulfide bridges) that immunospecifically bind TR4 (SEQ ID NO:1) or fragments or variants thereof, wherein the amino acid sequence of the heavy chain and the amino acid sequence of the light chain are the same as the amino acid sequence of a heavy chain and a light chain expressed by one or more scFvs or cell lines referred to in Table 1. In another embodiment, the invention provides antibodies (each consisting of two heavy chains and two light chains linked together by disulfide bridges to form an antibody) that immunospecifically bind TR4 or fragments or variants thereof, wherein the amino acid sequence of the heavy chain or the amino acid sequence of the light chain are the same as the amino acid sequence of a heavy chain or a light chain expressed by one or more scFvs or cell lines referred to in Table 1. Immunospecific binding to TR4 polypeptides may be determined by immunoassays known in the art or described herein for assaying specific antibody-antigen binding. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies that immunospecifically bind to TR4 are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies molecules, fragments and/or variants (e.g., SEQ ID NOs:54-65).

[0199] In one embodiment of the present invention, antibodies that immunospecifically bind to a TR4 or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the heavy chains expressed by at least one of the scFvs or cell lines referred to in Table 1 and/or any one of the light chains expressed by at least one of the scFvs or cell lines referred to in Table 1.

[0200] In another embodiment of the present invention, antibodies that immunospecifically bind to TR4 or a fragment or variant thereof, comprise a polypeptide

having the amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 and/or any one of the VL domains of at least one of the scFvs referred to in Table 1. In preferred embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and VL domain from a single scFv referred to in Table 1. In alternative embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and a VL domain from different scFvs referred to in Table 1. Molecules comprising, or alternatively consisting of, antibody fragments or variants of the VH and/or VL domains of at least one of the scFvs referred to in Table 1 that immunospecifically bind to a TR4 are also encompassed by the invention, as are nucleic acid molecules encoding these VH and VL domains, molecules, fragments and/or variants.

**[0201]** The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of TR4, wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VH CDRs contained in a VH domain of one or more scFvs referred to in Table 1. In particular, the invention provides antibodies that immunospecifically bind a TRAIL receptor, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VH CDR1 contained in a VH domain of one or more scFvs referred to in Table 1. In another embodiment, antibodies that immunospecifically bind TR4, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VH CDR2 contained in a VH domain of one or more scFvs referred to in Table 1. In a preferred embodiment, antibodies that immunospecifically bind TR4, comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VH CDR3 contained in a VH domain of one or more scFvs referred to in Table 1. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to TR4 or a TR4 fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants (e.g., SEQ ID NOs:54-65).

**[0202]** The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of TR4, wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VL CDRs contained in a VL domain of one or more scFvs

referred to in Table 1. In particular, the invention provides antibodies that immunospecifically bind TR4, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VL CDR1 contained in a VL domain of one or more scFvs referred to in Table 1. In another embodiment, antibodies that immunospecifically bind TR4, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VL CDR2 contained in a VL domain of one or more scFvs referred to in Table 1. In a preferred embodiment, antibodies that immunospecifically bind TR4, comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VL CDR3 contained in a VL domain of one or more scFvs referred to in Table 1. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to TR4 or a TR4 fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants (e.g., SEQ ID NOs:54-65).

**[0203]** The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) that immunospecifically bind to TR4 polypeptide or a fragment or variant of a TR4, wherein said antibodies comprise, or alternatively consist of, one, two, three, or more VH CDRs and one, two, three or more VL CDRs, as contained in a VH domain or VL domain of one or more scFvs referred to in Table 1. In particular, the invention provides for antibodies that immunospecifically bind to a polypeptide or polypeptide fragment or variant of TR4, wherein said antibodies comprise, or alternatively consist of, a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof, of the VH CDRs and VL CDRs contained in a VH domain or VL domain of one or more scFvs referred to in Table 1. In a preferred embodiment, one or more of these combinations are from the same scFv as disclosed in Table 1. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies, that immunospecifically bind to TR4 are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments or variants (e.g., SEQ ID NOs:54-65).

### **Nucleic Acid Molecules Encoding anti-TR4 Antibodies**

[0204] The present invention also provides for nucleic acid molecules, generally isolated, encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). In specific embodiments, the nucleic acid molecules encoding an antibody of the invention comprise, or alternatively consist of SEQ ID NOs:54-65 or fragments or variants thereof.

[0205] In a specific embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 and a VL domain having an amino acid sequence of VL domain of at least one of the scFvs referred to in Table 1. In another embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 or a VL domain having an amino acid sequence of a VL domain of at least one of the scFvs referred to in Table 1.

[0206] The present invention also provides antibodies that comprise, or alternatively consist of, variants (including derivatives) of the antibody molecules (e.g., the VH domains and/or VL domains) described herein, which antibodies immunospecifically bind to TR4 or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar

charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind TR4).

[0207] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations may be silent or neutral missense mutations, *i.e.*, have no, or little, effect on an antibody's ability to bind antigen. These types of mutations may be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (*e.g.*, ability to immunospecifically bind TR4) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0208] In a specific embodiment, an antibody of the invention (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds TR4 or a fragment or variant thereof, comprises, or alternatively consists of, an amino acid sequence encoded by a nucleotide sequence that hybridizes to a nucleotide sequence that is complementary to that encoding one of the VH or VL domains of one or more scFvs referred to in Table 1. under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45° C

followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3). Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0209] It is well known within the art that polypeptides, or fragments or variants thereof, with similar amino acid sequences often have similar structure and many of the same biological activities. Thus, in one embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to TR4 or fragments or variants of TR4, comprises, or alternatively consists of, a VH domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VH domain of at least one of the scFvs referred to in Table 1.

[0210] In another embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to TR4 or a fragment or variant of TR4, comprises, or alternatively consists of, a VL domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VL domain of at least one of the scFvs referred to in Table 1.

### **Methods of Producing Antibodies**

[0211] Antibodies in accordance with the invention are preferably prepared the utilization of a phage scFv display library. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

[0212] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (e.g., human or murine cDNA libraries of lymphoid tissues) or synthetic



cDNA libraries. The DNA encoding the VH and VL domains are joined together by an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to an antigen of interest (*i.e.*, a TRAIL receptor polypeptide or a fragment thereof) can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include, but are not limited to, those disclosed in Brinkman *et al.*, J. Immunol. Methods 182:41-50 (1995); Ames *et al.*, J. Immunol. Methods 184:177-186 (1995); Kettleborough *et al.*, Eur. J. Immunol. 24:952-958 (1994); Persic *et al.*, Gene 187 9-18 (1997); Burton *et al.*, Advances in Immunology 57:191-280(1994); PCT application No. PCT/GB91/O1 134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18719; WO 93/1 1236; WO 95/15982; WO 95/20401; WO97/13844; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,717; 5,780,225; 5,658,727; 5,735,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

**[0213]** For some uses, such as for in vitro affinity maturation of an antibody of the invention, it may be useful to express the VH and VL domains of one or more scFvs referred to in Table 1 as single chain antibodies or Fab fragments in a phage display library. For example, the cDNAs encoding the VH and VL domains of the scFvs referred to in Table 1 may be expressed in all possible combinations using a phage display library, allowing for the selection of VH/VL combinations that bind TR4 polypeptides with preferred binding characteristics such as improved affinity or improved off rates. Additionally, VH and VL segments - the CDR regions of the VH and VL domains of the scFvs referred to in Table 1, in particular, may be mutated in vitro. Expression of VH and VL domains with "mutant" CDRs in a phage display library allows for the selection of VH/VL combinations that bind TR4 polypeptides with preferred binding characteristics such as improved affinity or improved off rates.

### **Additional Methods of Producing Antibodies**

[0214] Antibodies of the invention (including antibody fragments or variants) can be produced by any method known in the art. For example, it will be appreciated that antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells or to generate phage display libraries, for example. Additionally, polypeptide antibodies of the invention may be chemically synthesized or produced through the use of recombinant expression systems.

[0215] One way to produce the antibodies of the invention would be to clone the VH and/or VL domains of the scFvs referred to in Table 1. In order to isolate the VH and VL domains from bacteria transfected with a vector containing the scFv, PCR primers complementary to VH or VL nucleotide sequences (See Example 5), may be used to amplify the VH and VL sequences. The PCR products may then be cloned using vectors, for example, which have a PCR product cloning site consisting of a 5' and 3' single T nucleotide overhang, that is complementary to the overhanging single adenine nucleotide added onto the 5' and 3' end of PCR products by many DNA polymerases used for PCR reactions. The VH and VL domains can then be sequenced using conventional methods known in the art. Alternatively, the VH and VL domains may be amplified using vector specific primers designed to amplify the entire scFv, (i.e. the VH domain, linker and VL domain.)

[0216] The cloned VH and VL genes may be placed into one or more suitable expression vectors. By way of non-limiting example, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site may be used to amplify the VH or VL sequences. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains may be cloned into vectors expressing the appropriate immunoglobulin constant region, *e.g.*, the human IgG1 or IgG4 constant region for VH domains, and the human kappa or lambda constant regions for kappa and lambda VL domains, respectively. Preferably, the vectors for expressing the VH or VL domains comprise a promoter suitable to direct expression of the heavy and light chains in the chosen expression system, a secretion signal, a cloning site for the immunoglobulin variable domain, immunoglobulin constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into a single

vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art (See, for example, Guo et al., J. Clin. Endocrinol. Metab. 82:925-31 (1997), and Ames et al., J. Immunol. Methods 184:177-86 (1995) which are herein incorporated in their entirety by reference).

[0217] The invention provides polynucleotides comprising, or alternatively consisting of, a nucleotide sequence encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). The invention also encompasses polynucleotides that hybridize under high stringency, or alternatively, under intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides complementary to nucleic acids having a polynucleotide sequence that encodes an antibody of the invention or a fragment or variant thereof.

[0218] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. If the amino acid sequences of the VH domains, VL domains and CDRs thereof, are known, nucleotide sequences encoding these antibodies can be determined using methods well known in the art, *i.e.*, the nucleotide codons known to encode the particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody, of the invention. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0219] Alternatively, a polynucleotide encoding an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells or Epstein Barr virus transformed B cell lines that express an antibody of the invention) by PCR amplification using synthetic

primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0220] Once the nucleotide sequence of the antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0221] In a specific embodiment, VH and VL domains of one or more scFvs referred to in Table 1, or fragments or variants thereof, are inserted within framework regions using recombinant DNA techniques known in the art. In a specific embodiment, one, two, three, four, five, six, or more of the CDRs of VH and/or VL domains of one or more scFvs referred to in Table 1, or fragments or variants thereof, is inserted within framework regions using recombinant DNA techniques known in the art. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia *et al.*, J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions, the contents of which are hereby incorporated by reference in its entirety). Preferably, the polynucleotides generated by the combination of the framework regions and CDRs encode an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically binds to a TRAIL receptor. Preferably, as discussed *supra*, polynucleotides encoding variants of antibodies or antibody fragments having one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions do not significantly alter binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region

cysteine residues participating in an intrachain disulfide bond to generate antibody molecules, or antibody fragments or variants, lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and fall within the ordinary skill of the art.

[0222] The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

[0223] An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease.

[0224] Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Monoclonal antibodies and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as cancer, which require repeated antibody administrations.

[0225] One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including

human antigens. Using the hybridoma technology, antigen-specific human Monoclonal antibodies with the desired specificity could be readily produced and selected.

[0226] This general strategy was demonstrated in connection with the generation of the first XenoMouse™ strains as published in 1994. *See* Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACS) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. Id. The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human monoclonal antibodies. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. *See* Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J Exp. Med.* 188:483-495 (1998), Green, *Journal of Immunological Methods* 231:11-23 (1999) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

[0227] Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/710,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 0-8/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/471,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* 15:146-156

(1997) and Green and Jakobovits *J Exp. Med.* 188:483-495 (1998). *See also* European Patent No., EP 0 471 151 B1, grant published June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

**[0228]** Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies against TR4 polypeptides in order to vitiate concerns and/or effects of HAMA or HACA responses.

**[0229]** Monoclonal antibodies specific for TR4 polypeptides may be prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 571-681 (1981)). Briefly, XenoMouse™ mice may be immunized with TR4 polypeptides. After immunization, the splenocytes of such mice were extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the TR4 polypeptides.

**[0230]** For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human patients. *See also*, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50435, WO 98/24893, WO98/16654, WO 96/34096, WO 96/35735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. In a specific embodiment, antibodies of the present invention comprise one or more VH and VL

domains of the invention and constant regions from another immunoglobulin molecule, preferably a human immunoglobulin molecule. In a specific embodiment, antibodies of the present invention comprise one or more CDRs corresponding to the VH and VL domains of the invention and framework regions from another immunoglobulin molecule, preferably a human immunoglobulin molecule. In other embodiments, an antibody of the present invention comprises one, two, three, four, five, six or more VL CDRs or VH CDRs corresponding to one or more of the VH or VL domains of one or more scFvs referred to in Table 1, or fragments or variants thereof, and framework regions (and, optionally one or more CDRs not derived from the antibodies expressed by scFvs referred to in Table 1) from a human immunoglobulin molecule. In a preferred embodiment, an antibody of the present invention comprises a VH CDR3, VL CDR3, or both, corresponding to the same scFv, or different scFvs selected from the scFvs referred to in Table 1, or fragments or variants thereof, and framework regions from a human immunoglobulin.

[0231] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a human antibody and a non-human (e.g., murine) immunoglobulin constant region or vice versa. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, *Science* 229:1202 (1985); Oi *et al.*, *BioTechniques* 4:214 (1986); Gillies *et al.*, *J. Immunol. Methods* 125:191-202 (1989); U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Chimeric antibodies comprising one or more CDRs from human species and framework regions from a non-human immunoglobulin molecule (e.g., framework regions from a murine, canine or feline immunoglobulin molecule) (or vice versa) can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka *et al.*, *Protein Engineering* 7(6):805-814 (1994); Roguska *et al.*, *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,352). In a preferred embodiment, chimeric antibodies comprise a human CDR3 having an amino acid sequence of any one of the VH CDR3s or VL CDR3s of a VH or VL domain of one or more of the scFvs referred to in Table 1, or a variant thereof, and non-human framework regions or human framework regions different from those of



the frameworks in the corresponding scFv disclosed in Table 1. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, Nature 352:323 (1988), which are incorporated herein by reference in their entireties.)

[0232] Intrabodies are antibodies, often scFvs, that are expressed from a recombinant nucleic acid molecule and engineered to be retained intracellularly (*e.g.*, retained in the cytoplasm, endoplasmic reticulum, or periplasm). Intrabodies may be used, for example, to ablate the function of a protein to which the intrabody binds. The expression of intrabodies may also be regulated through the use of inducible promoters in the nucleic acid expression vector comprising the intrabody. Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen *et al.*, *Hum. Gene Ther.* 5:595-601 (1994); Marasco, W.A., *Gene Ther.* 4:11-15 (1997); Rondon and Marasco, *Annu. Rev. Microbiol.* 51:257-283 (1997); Proba *et al.*, *J. Mol. Biol.* 275:245-253 (1998); Cohen *et al.*, *Oncogene* 17:2445-2456 (1998); Ohage and Steipe, *J. Mol. Biol.* 291:1119-1128 (1999); Ohage *et al.*, *J. Mol. Biol.* 291:1129-1134 (1999); Wirtz and Steipe, *Protein Sci.* 8:2245-2250 (1999); Zhu *et al.*, *J. Immunol. Methods* 231:207-222 (1999); and references cited therein.

[0233] Recombinant expression of an antibody of the invention (including antibody fragments or variants thereof (*e.g.*, a heavy or light chain of an antibody of the invention), requires construction of an expression vector(s) containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule (*e.g.*, a whole antibody, a heavy or light chain of an antibody, or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable domain)), of the invention has been obtained, the vector(s) for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods

include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention (e.g., a whole antibody, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody, or a portion thereof, or a heavy or light chain CDR, a single chain Fv, or fragments or variants thereof), operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464, the contents of each of which are hereby incorporated by reference in its entirety) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy chain, the entire light chain, or both the entire heavy and light chains.

[0234] The expression vector(s) is(are) transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing polynucleotide(s) encoding an antibody of the invention (e.g., whole antibody, a heavy or light chain thereof, or portion thereof, or a single chain antibody, or a fragment or variant thereof), operably linked to a heterologous promoter. In preferred embodiments, for the expression of entire antibody molecules, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0235] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include, but are not limited to, bacteriophage particles engineered to express antibody fragments or variants thereof (single chain antibodies), microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell

systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3, NS0 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, Gene 45:101 (1986); Cockett *et al.*, Bio/Technology 8:2 (1990); Bebbington *et al.*, Bio/Techniques 10:169 (1992); Keen and Hale, Cytotechnology 18:207 (1996)). These references are incorporated in their entireties by reference herein.

[0236] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, EMBO 1. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0237] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) may be used as a vector to express foreign genes. The virus grows in

*Spodoptera frugiperda* cells. Antibody coding sequences may be cloned individually into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter).

[0238] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner *et al.*, Methods in Enzymol. 153:51-544 (1987)).

[0239] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERY, BHK, HeLa, COS, NSO, MDCK, 293, 3T3, W138, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and HsS78Bst.

[0240] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[0241] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, Cell 22:8 17 (1980)) genes can be employed in tk-, hgp<sup>rt</sup>- or ap<sup>rt</sup>- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, Natl. Acad. Sci. USA 77:357 (1980); O'Hare *et al.*, Proc. Natl. Acad. Sci. USA 78:1527 (1981)); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62: 191-217 (1993); TIB TECH 11(5):155-2 15 (May, 1993)); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), Current

Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0242] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells" in *DNA Cloning, Vol.3*. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the coding sequence of the antibody, production of the antibody will also increase (Crouse *et al.*, Mol. Cell. Biol. 3:257 (1983)).

[0243] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

[0244] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain is preferably placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot,

Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2 197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

**[0245]** Once an antibody molecule of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) has been chemically synthesized or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, or more generally, a protein molecule, such as, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention may be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

#### **Characterization of anti-TR4 Antibodies**

**[0246]** Antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may also be described or specified in terms of their binding to TR4 polypeptides or fragments or variants of TR4 polypeptides. In specific embodiments, antibodies of the invention bind TR4 polypeptides, or fragments or variants thereof, with a dissociation constant or  $K_D$  of less than or equal to  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M, or  $10^{-5}$  M. More preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with a dissociation constant or  $K_D$  less than or equal to  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M, or  $10^{-8}$  M. Even more preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with a dissociation constant or  $K_D$  less than or equal to  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. The invention encompasses antibodies that bind TR4 polypeptides with a dissociation constant or  $K_D$  that is within any one of the ranges that are between each of the individual recited values.

**[0247]** In specific embodiments, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with an off rate ( $k_{off}$ ) of less than or equal to  $5 \times 10^{-2} \text{ sec}^{-1}$ ,  $10^{-2} \text{ sec}^{-1}$ ,  $5 \times 10^{-3} \text{ sec}^{-1}$  or  $10^{-3} \text{ sec}^{-1}$ . More preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with an off rate ( $k_{off}$ ) less than or equal

to  $5 \times 10^{-4} \text{ sec}^{-1}$ ,  $10^{-4} \text{ sec}^{-1}$ ,  $5 \times 10^{-5} \text{ sec}^{-1}$ , or  $10^{-5} \text{ sec}^{-1}$   $5 \times 10^{-6} \text{ sec}^{-1}$ ,  $10^{-6} \text{ sec}^{-1}$ ,  $5 \times 10^{-7} \text{ sec}^{-1}$  or  $10^{-7} \text{ sec}^{-1}$ . The invention encompasses antibodies that bind TR4 polypeptides with an off rate ( $k_{\text{off}}$ ) that is within any one of the ranges that are between each of the individual recited values.

**[0248]** In other embodiments, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with an on rate ( $k_{\text{on}}$ ) of greater than or equal to  $10^3 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $10^4 \text{ M}^{-1} \text{ sec}^{-1}$  or  $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$ . More preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with an on rate ( $k_{\text{on}}$ ) greater than or equal to  $10^5 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$ ,  $10^6 \text{ M}^{-1} \text{ sec}^{-1}$ , or  $5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$  or  $10^7 \text{ M}^{-1} \text{ sec}^{-1}$ . The invention encompasses antibodies that bind TR4 polypeptides with on rate ( $k_{\text{on}}$ ) that is within any one of the ranges that are between each of the individual recited values.

**[0249]** The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) immunospecifically bind to a polypeptide or polypeptide fragment or variant of human TR4 polypeptides (SEQ ID NOS:1). In another embodiment, the antibodies of the invention immunospecifically bind to a polypeptide or polypeptide fragment or variant of simian TR4 polypeptides. In yet another embodiment, the antibodies of the invention immunospecifically bind to a polypeptide or polypeptide fragment or variant of murine TR4 polypeptides. In one embodiment, the antibodies of the invention bind immunospecifically to human and simian TR4 polypeptides. In another embodiment, the antibodies of the invention bind immunospecifically to human TR4 polypeptides and murine TR4 polypeptides. More preferably, antibodies of the invention, preferentially bind to human TR4 polypeptides compared to murine TR4 polypeptides.

**[0250]** In preferred embodiments, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), immunospecifically bind to TR4 polypeptides and do not cross-react with any other antigens. In preferred embodiments, the antibodies of the invention immunospecifically bind to TR4 polypeptides (e.g., SEQ ID NOS:1 or fragments or variants thereof) and do not cross-react with one or more additional members of the Tumor Necrosis Factor Tumor Necrosis Factor Receptor Family polypeptides (e.g., TR1, TR5, TR10 BCMA, TACI, CD30, CD27, OX40, 4-1BB, CD40, NGFR, TNFR1, TNFR2, Fas, and NGFR).



[0251] In another embodiment, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), immunospecifically bind to TR4 polypeptides and cross-react with other antigens. In other embodiments, the antibodies of the invention immunospecifically bind to TR4 polypeptides (e.g., SEQ ID NOS:1 or fragments or variants thereof) and cross-react with one or more additional members of the Tumor Necrosis Factor Receptor Family polypeptides (e.g., TR1, TR5, TR10 BCMA, TACI, CD30, CD27, OX40, 4-1BB, CD40, NGFR, TNFR1, TNFR2, Fas, and NGFR).

[0252] In a preferred embodiment, antibodies of the invention preferentially bind TR4 (SEQ ID NO:1), or fragments and variants thereof relative to their ability to bind TR1, TR5, TR7, or TR10 (SEQ ID NOS:2-5) or fragments or variants thereof. In other preferred embodiments, the antibodies of the invention preferentially bind to TR4 and TR7 (SEQ ID NOS:1 and 3), or fragments and variants thereof relative to their ability to bind TR1, TR5 or TR10 (SEQ ID NOS:5, 2 and 4) or fragments or variants thereof. In other preferred embodiments, the antibodies of the invention bind TR1, TR4, TR5, TR7 and TR10 (SEQ ID NOS:5, 1, 2, 3 and 4). An antibody's ability to preferentially bind one antigen compared to another antigen may be determined using any method known in the art.

[0253] By way of non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a dissociation constant ( $K_D$ ) that is less than the antibody's  $K_D$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity (i.e.,  $K_D$ ) that is at least one order of magnitude less than the antibody's  $K_D$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity (i.e.,  $K_D$ ) that is at least two orders of magnitude less than the antibody's  $K_D$  for the second antigen.

[0254] In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an off rate ( $k_{off}$ ) that is less than the antibody's  $k_{off}$  for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a  $k_{off}$  that is at least one order of magnitude less than the antibody's  $k_{off}$  for the second antigen. In another non-limiting embodiment, an antibody may be considered

to bind a first antigen preferentially if it binds said first antigen with a  $k_{\text{off}}$  that is at least two orders of magnitude less than the antibody's  $k_{\text{off}}$  for the second antigen.

[0255] The invention also encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that have one or more of the same biological characteristics as one or more of the antibodies described herein. By "biological characteristics" is meant, the in vitro or in vivo activities or properties of the antibodies, such as, for example, the ability to bind to TR4 polypeptides (e.g., membrane-embedded TRAIL receptors), the ability to stimulate TR4 mediated biological activity (e.g., to stimulate apoptosis of TR4 expressing cells, see Example 4); the ability to substantially block TR4 ligand (e.g. TRAIL (SEQ ID NO:66), also known as AIM-I, International Application No. WO 97/35899 and U.S. Patent Application 5,771,223), or a fragment, variant or fusion protein thereof, binding to TRAIL receptor, see Example 3; or the ability to upregulate TR4 expression on the surface of cells. Other biological activities that antibodies against TR4 polypeptides may have, include, but are not limited to, the ability to inhibit TR4 mediated biological activity (e.g., to inhibit apoptosis of TR4 expressing cells) or the ability to downregulate TR4 expression on the surface of cells. Optionally, the antibodies of the invention will bind to the same epitope as at least one of the antibodies specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

[0256] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that stimulate TR4 mediated biological activities. In one embodiment, an antibody that stimulates TR4 mediated biological activities comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1, or fragment or variant thereof. In a specific embodiment, an antibody that stimulates TR4 mediated biological activities comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1, or fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0257] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that stimulate apoptosis of TR4 expressing cells (see Example 4). In one embodiment, an antibody that stimulates apoptosis of TR4 expressing cells comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1, or

fragment or variant thereof. In a specific embodiment, an antibody that stimulates apoptosis of TR4 expressing cells comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1, or fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

**[0258]** In preferred embodiments, the present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that stimulate apoptosis of TR4 expressing cells equally well in the presence or absence of antibody cross-linking reagents, such as for example anti-Ig Fc reagents cells (See, for example, Example 4). In a specific embodiment, antibodies of the present invention stimulate apoptosis of HeLa cells, equally well in the presence or absence of an anti-Ig Fc antibody cross-linking reagent. In another specific embodiment, antibodies of the present invention stimulate apoptosis of HeLa cells, equally well in the presence or absence of an anti-Ig Fc antibody cross-linking reagent in the presence of 2 micrograms/milliliter of cycloheximide. In another embodiment, antibodies of the present invention stimulate apoptosis of SW480 cells, equally well in the presence or absence of an anti Ig Fc antibody cross-linking reagent. In another specific embodiment, antibodies of the present invention stimulate apoptosis of SW480 cells, equally well in the presence or absence of an anti-Ig Fc antibody cross-linking reagent in the presence of 2 micrograms/milliliter of cycloheximide.

**[0259]** In other preferred embodiments, the present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that stimulate apoptosis of TR4 expressing cells at least as well as an equal concentration (in terms of, for example, nanograms/milliliter) of TRAIL polypeptide (including TRAIL polypeptide fragments, variants or fusion proteins) stimulates apoptosis of TR4 expressing cells (See, for example, Example 4). In a specific embodiment, antibodies of the invention stimulate apoptosis of TR4 expressing cells better than an equal concentration (in terms of, for example, nanograms/milliliter) of TRAIL polypeptide (including TRAIL polypeptide fragments, variants or fusion proteins) stimulates apoptosis of TR4 expressing cells. In a specific embodiment, antibodies of the invention stimulate apoptosis of HeLa cells better than an equal concentration (in terms of, for example, nanograms/milliliter) of TRAIL polypeptide (including TRAIL polypeptide fragments, variants or fusion proteins) stimulates apoptosis of TR4 expressing cells. In another specific embodiment, antibodies of the present invention stimulate apoptosis of

HeLa cells better than an equal concentration (in terms of, for example, nanograms/milliliter) of TRAIL polypeptide (including TRAIL polypeptide fragments, variants or fusion proteins) stimulates apoptosis of TR4 expressing cells in the presence of 2 micrograms/milliliter of cycloheximide.

**[0260]** In other preferred embodiments, the present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that stimulate more apoptosis of TR4 expressing cells when administered in combination with a chemotherapeutic drug, than either the chemotherapeutic drug or the antibodies alone stimulate apoptosis of receptor expressing cells. In specific embodiments, antibodies of the present invention, stimulate more apoptosis of TR4 expressing cells when administered in combination with Topotecan, than either Topotecan or the antibodies alone stimulate apoptosis of receptor expressing cells. In specific embodiments, antibodies of the present invention, stimulate more apoptosis of TR4 expressing cells when administered in combination with cycloheximide, than either cycloheximide or the antibodies alone stimulate apoptosis of receptor expressing cells.

**[0261]** The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the binding of TRAIL to a TR4 polypeptide (see Example 3). In one embodiment, an antibody that blocks or inhibits the binding of TRAIL to TR4 comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1, or fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the binding of TRAIL to TR4 comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1, or fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

**[0262]** The present invention also provides for fusion proteins comprising, or alternatively consisting of, an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that immunospecifically binds to TR4, and a heterologous polypeptide. Preferably, the heterologous polypeptide to which the antibody is fused to is useful for function or is useful to target the TR4 expressing cells. In specific embodiments the invention encompasses bispecific antibodies that in which one antibody binding site is specific for TR4 and the second antibody binding site is specific for a heterologous polypeptide such as TR7 or a tumor specific antigen. In an

alternative preferred embodiment, the heterologous polypeptide to which the antibody is fused to is useful to target the antibody to a tumor cell. In one embodiment, a fusion protein of the invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one or more of the VH domains of an antibody of the invention or the amino acid sequence of any one or more of the VL domains of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. In another embodiment, a fusion protein of the present invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one, two, three, or more of the VH CDRs of an antibody of the invention, or the amino acid sequence of any one, two, three, or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. In a preferred embodiment, the fusion protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of, a VH CDR3 of an antibody of the invention, or fragment or variant thereof, and a heterologous polypeptide sequence, which fusion protein immunospecifically binds to TR4. In another embodiment, a fusion protein comprises, or alternatively consists of a polypeptide having the amino acid sequence of at least one VH domain of an antibody of the invention and the amino acid sequence of at least one VL domain of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, the VH and VL domains of the fusion protein correspond to a single antibody (or scFv or Fab fragment) of the invention. In yet another embodiment, a fusion protein of the invention comprises, or alternatively consists of a polypeptide having the amino acid sequence of any one, two, three or more of the VH CDRs of an antibody of the invention and the amino acid sequence of any one, two, three or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, two, three, four, five, six, or more of the VHCDR(s) or VLCDR(s) correspond to single antibody (or scFv or Fab fragment) of the invention. Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention.

**[0263]** Antibodies of the present invention (including antibody fragments or variants thereof) may be characterized in a variety of ways. In particular, antibodies and related molecules of the invention may be assayed for the ability to immunospecifically bind to TR4 or a fragment or variant of TR4, using techniques described herein or routinely modifying techniques known in the art. Assays for the ability of the antibodies of the

invention to immunospecifically bind TR4 or a fragment or variant of TR4, may be performed in solution (*e.g.*, Houghten, *Bio/Techniques* 13:412-421(1992)), on beads (*e.g.*, Lam, *Nature* 354:82-84 (1991)), on chips (*e.g.*, Fodor, *Nature* 364:555-556 (1993)), on bacteria (*e.g.*, U.S. Patent No. 5,223,409), on spores (*e.g.*, Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (*e.g.*, Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869 (1992)) or on phage (*e.g.*, Scott and Smith, *Science* 249:386-390 (1990); Devlin, *Science* 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87:7178-7182 (1990); and Felici, *J. Mol. Biol.* 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). Antibodies that have been identified to immunospecifically bind to TR4 or a fragment or variant of TR4 can then be assayed for their specificity and affinity for TR4 or a fragment or variant of TR4, using or routinely modifying techniques described herein or otherwise known in the art.

**[0264]** The antibodies of the invention may be assayed for immunospecific binding to TR4 polypeptides and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as BIAcore analysis (See, *e.g.*, Example 2), FACS (fluorescence activated cell sorter) analysis, immunofluorescence, immunocytochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, western blots, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al., eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

**[0265]** ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound antibodies or non-specifically bound antibodies, and detecting the presence of the antibodies specifically bound to the antigen coating the

well. In ELISAs, the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Alternatively, the antigen need not be directly coated to the well; instead the ELISA plates may be coated with an anti-Ig Fc antibody, and the antigen in the form of a TRAIL receptor-Fc fusion protein, may be bound to the anti-Ig Fc coated to the plate. This may be desirable so as to maintain the antigen protein (e.g., the TR4 polypeptides) in a more native conformation than it may have when it is directly coated to a plate. In another alternative, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

**[0266]** The binding affinity of an antibody (including an scFv or other molecule comprising, or alternatively consisting of, antibody fragments or variants thereof) to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., antigen labeled with  $^3\text{H}$  or  $^{125}\text{I}$ ), or fragment or variant thereof with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention for TR4 and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, a TR4 polypeptide is incubated with an antibody of the present invention conjugated to a labeled compound (e.g., compound labeled with  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second anti-TR4 antibody. This kind of competitive assay between two antibodies, may also be used to determine if two antibodies bind the same or different epitopes.

**[0267]** In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies (including antibody fragments or variants thereof) to a TRAIL receptor, or fragments of a TRAIL receptor. BIAcore kinetic analysis comprises

analyzing the binding and dissociation of antibodies from chips with immobilized TRAIL receptors on their surface as described in detail in Example 2.

**[0268]** Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40 degrees C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

**[0269]** Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.



### Antibody Conjugates

[0270] The present invention encompasses antibodies (including antibody fragments or variants thereof), recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous polypeptide (or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, antibodies of the invention may be used to target heterologous polypeptides to particular cell types (*e.g.*, cancer cells), either *in vitro* or *in vivo*, by fusing or conjugating the heterologous polypeptides to antibodies of the invention that are specific for particular cell surface antigens or which bind antigens that bind particular cell surface receptors. Antibodies of the invention may also be fused to albumin (including but not limited to recombinant human serum albumin (see, *e.g.*, U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (*i.e.*, amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (*e.g.*, immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention. Such fusion proteins may, for example, facilitate purification and may increase half-life *in vivo*. Antibodies fused or conjugated to heterologous polypeptides may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, Harbor *et al.*, *supra*, and PCT publication WO 93/2 1232; EP 439,095; Naramura *et al.*, *Immunol. Lett.* 39:91-99 (1994);

U.S. Patent 5,474,981; Gillies *et al.*, PNAS 89:1428-1432 (1992); Fell *et al.*, J. Immunol. 146:2446-2452 (1991), which are incorporated by reference in their entireties.

[0271] The present invention further includes compositions comprising, or alternatively consisting of, heterologous polypeptides fused or conjugated to antibody fragments. For example, the heterologous polypeptides may be fused or conjugated to a Fab fragment, Fd fragment, Fv fragment, F(ab)<sub>2</sub> fragment, or a portion thereof. Methods for fusing or conjugating polypeptides to antibody portions are known in the art. See, *e.g.*, U.S. Patent Nos. 5,356,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 9 1/06570; Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA 88: 10535-10539 (1991); Zheng *et al.*, J. Immunol. 154:5590-5600 (1995); and Vil *et al.*, Proc. Natl. Acad. Sci. USA 89:11357- 11341 (1992) (said references incorporated by reference in their entireties).

[0272] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), such methods can be used to generate antibodies with altered activity (*e.g.*, antibodies with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten *et al.*, Curr. Opinion Biotechnol. 8:724-35 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, *et al.*, J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, polynucleotides encoding antibodies of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more portions of a polynucleotide encoding an antibody which portions immunospecifically bind to TR4 may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0273] Moreover, the antibodies of the present invention (including antibody fragments or variants thereof), can be fused to marker sequences, such as a polypeptides to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine polypeptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259

Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, Cell 37:767 (1984)) and the FLAG® tag (Stratagene, La Jolla, CA).

[0274] The present invention further encompasses antibodies (including antibody fragments or variants thereof), conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor or prognose the development or progression of a tumor as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include, but are not limited to, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include, but are not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include, but are not limited to, streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include, but are not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes, but is not limited to, luminol; examples of bioluminescent materials include, but are not limited to, luciferase, luciferin, and aequorin; and examples of suitable radioactive material include, but are not limited to, iodine ( $^{121}\text{I}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{111}\text{In}$ ,  $^{112}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{115\text{m}}\text{In}$ ), technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{135}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ , and  $^{97}\text{Ru}$ .

[0275] Further, an antibody of the invention (including an scFv or other molecule comprising, or alternatively consisting of, antibody fragments or variants thereof), may be coupled or conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters such as, for example,  $^{213}\text{Bi}$ , or other radioisotopes such as, for example,  $^{103}\text{Pd}$ ,  $^{135}\text{Xe}$ ,  $^{131}\text{I}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ ,  $^{90}\text{Y}$ ,  $^{117}\text{Tm}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$  and  $^{166}\text{Ho}$ . In specific embodiments, an antibody or fragment thereof is attached to macrocyclic chelators that chelate radiometal ions, including but not limited to,  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{166}\text{Ho}$ , and  $^{153}\text{Sm}$ , to polypeptides. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the an antibody of the invention or fragment thereof via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al., Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety.

[0276] A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, thymidine kinase, endonuclease, RNase, and puromycin and fragments, variants or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0277] Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional

conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,711; 5,696,239; 5,652,371; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter and Chloramine-T reaction).

[0278] The antibodies of the invention which are conjugates can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, but are not limited to, for example, a toxin such as abrin, ricin A, alpha toxin, pseudomonas exotoxin, or diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF-alpha, TNF-beta, AIM I (see, International Publication No. WO 97/35899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (see, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or other growth factors.

[0279] Antibodies of the invention (including antibody fragments or variants thereof), may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0280] Techniques for conjugating a therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal*

Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0281] Alternatively, an antibody of the invention can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0282] An antibody of the invention (including an other molecules comprising, or alternatively consisting of, an antibody fragment or variant thereof), with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### **Uses of Antibodies of the Invention**

[0283] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of TR4 polypeptides in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

### **Immunophenotyping**

[0284] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples (See, for example, Example 4). The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types, particularly of tumors and cancer cells. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

[0285] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### **Epitope Mapping**

[0286] The present invention provides antibodies (including antibody fragments or variants thereof), that can be used to identify epitopes of a TR4 polypeptide. In particular, the antibodies of the present invention can be used to identify epitopes of a human TR4 polypeptide (e.g., SEQ ID NOS:1) or a TR4 polypeptide expressed on human cells; a murine TR4 or a TR4 polypeptide expressed on murine cells; a rat TR4 polypeptide receptor or a TR4 polypeptide expressed on rat cells; or a monkey TR4 polypeptide or a TR4 polypeptide expressed on monkey cells, using techniques described herein or otherwise known in the art. Fragments which function as epitopes may be produced by any conventional means. (See, *e.g.*, Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,711,211.) Identified epitopes of antibodies of the present invention may, for example, be used as vaccine candidates, i.e., to immunize an individual to elicit antibodies against the naturally occurring forms of TR4 polypeptides.

### **Diagnostic Uses of Antibodies**

[0287] Labeled antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which specifically bind to a TR4 polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor diseases and/or disorders. In specific embodiments, labeled antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which specifically bind to a TR4 polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of TR4 .

[0288] The invention provides for the detection of expression of a TR4 polypeptide comprising: (a) assaying the expression of a TR4 polypeptide in a biological sample from an individual using one or more antibodies of the invention that immunospecifically binds

to TR4; and (b) comparing the level of TR4 polypeptide in the biological sample with a standard level of TR4 polypeptide, (*e.g.*, the level in normal biological samples).

[0289] The invention provides for the detection of aberrant expression of a TR4 polypeptide comprising: (a) assaying the expression of a TR4 polypeptide in a biological sample from an individual using one or more antibodies of the invention that immunospecifically binds to TR4; and (b) comparing the level of a TR4 polypeptide in the biological sample with a standard level of a TR4 polypeptide, *e.g.*, in normal biological samples, whereby an increase or decrease in the assayed level of a TR4 polypeptide compared to the standard level of a TR4 polypeptide is indicative of aberrant expression.

[0290] By “biological sample” is intended any fluids and/or cells obtained from an individual, body fluid, body tissue, body cell, cell line, tissue culture, or other source which may contain a TR4 polypeptide protein or mRNA. Body fluids include, but are not limited to, sera, plasma, urine, synovial fluid, spinal fluid, saliva, and mucous. Tissues samples may be taken from virtually any tissue in the body. Tissue samples may also be obtained from autopsy material. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0291] Antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which specifically bind to a TR4 polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor cancers and other hyperproliferative disorders, and/or diseases or conditions associated therewith. The invention provides for the detection of aberrant expression of TR4 polypeptide comprising: (a) assaying the expression of TR4 polypeptide in a biological sample from an individual using one or more antibodies of the invention that immunospecifically binds to a TR4 polypeptide; and (b) comparing the level of a TR4 polypeptide with a standard level of TR4 polypeptide, *e.g.*, in normal biological samples, whereby an increase or decrease in the assayed level of TR4 polypeptide compared to the standard level of TR4 polypeptide is indicative of a cancer and/or a hyperproliferative disorder.

[0292] TRAIL has been shown in some instances to selectively kill tumor cells (See, for example, Oncogene 19:3363-71 (2000)). This may be a result of differential expression of TRAIL receptors on normal and cancerous cells. Thus, in specific



embodiments, an increase in the assayed level of a TR4 polypeptide is indicative of a cancer and/or a hyperproliferative disorder.

[0293] Other reports suggest that decreased TR4 expression by tumor cells may be a mechanism by which tumor cells evade the immune system (See, for example, Int. J. Oncol. 16:917-25 (2000)) Thus, in other specific embodiments, a decrease in the assayed level of TR4 polypeptide is indicative of a cancer and/or a hyperproliferative disorder.

[0294] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of TR4 in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically binds to a TR4 polypeptide; b) waiting for a time interval following the administering for permitting the labeled antibody to preferentially concentrate at sites in the subject where TR4 polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody or fragment thereof above the background level and above or below the level observed in a person without the disease or disorder indicates that the subject has a particular disease or disorder associated with aberrant expression of TR4 polypeptide. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0295] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99</sup>Tc. The labeled antibody will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0296] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled

molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment, the time interval following administration is 5 to 20 days or 5 to 10 days.

[0297] In one embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0298] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0299] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### **Therapeutic Uses of Antibodies**

[0300] One or more antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to TR4 may be used locally or systemically in the body as a therapeutic. The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) to an animal, preferably a mammal, and most preferably a human, for preventing or treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention and nucleic acids encoding

antibodies (and anti-idiotypic antibodies) of the invention as described herein. In one embodiment, the antibodies of the invention can be used to treat, ameliorate or prevent diseases, disorders or conditions, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein. In certain embodiments, properties of the antibodies of the present invention, as detailed in the Examples below, make the antibodies better therapeutic agents than previously described TR4 binding antibodies.

### **Therapeutic Uses of Antibodies for Treating Cancers**

**[0301]** In highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate cancer. In specific embodiments, antibodies of the invention are used to inhibit the progression or metastasis of cancers and other related disorders. Cancers and related disorders, include, but are not limited to, colon cancer, cervical cancer, leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma,

medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

**[0302]** In highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate renal cancer.

**[0303]** In highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate melanoma.

**[0304]** In highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate cancers of the liver such as hepatomas.

**[0305]** In highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate cancers of the central nervous system such as medulloblastoma, neuroblastoma, and glioblastoma.

**[0306]** It has been demonstrated, in accordance with the present invention that the expression of TRAIL receptor TR4 on lung carcinoma tissue, bladder carcinoma tissue and Ovarian carcinoma tissue. Additionally, it has been demonstrated, in accordance with the present invention that TRAIL receptor TR4 is expressed on primary breast, colon, lung, and stomach tumor tissue. (See Example 9). Thus, in highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat lung cancer. In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat bladder cancer. In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat ovarian cancer. In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat breast cancer. In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat colon cancer and/or colorectal cancer. In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat stomach cancer.

[0307] In other highly preferred embodiments, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat, prevent or ameliorate renal cancer, melanoma, pancreatic cancer and cancers of the liver such as hepatomas.

[0308] In another embodiment, antibodies of the invention that bind TR4 and stimulate apoptosis of TR4 expressing cells are used to treat diseases and/or disorders associated with increased cell survival, or the inhibition of apoptosis, including cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), information graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the antibodies and antibody compositions of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above. In preferred embodiments the antibodies and antibody compositions of the invention are not hepatotoxic, *in vitro* or *in vivo*.

#### **Additional Therapeutic Uses of Antibodies**

[0309] In another embodiment, the invention provides methods and compositions for inhibiting the growth of or killing TR4 expressing cells, comprising, or alternatively consisting of, administering to an animal in which such inhibition of growth or killing of TR4 expressing cells is desired, antibody or antibody compositions of the invention (e.g., antibody fragments and variants, antibody mixtures, antibody multimers, fusion proteins of the invention, and antibodies in combination with other therapeutic compounds such as chemotherapeutic agents) in an amount effective to inhibit the growth of or kill TR4 expressing cells.

[0310] In one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand (especially TRAIL (SEQ ID NO:66)), which

involves administering to a cell which expresses a TR4 polypeptide an effective amount of an antibody of the invention, preferably an agonistic anti-TR4 antibody, capable of inducing or increasing TR4 mediated signaling. Preferably, TR4 mediated signaling is increased or induced by an antibody of the invention to treat a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited.

[0311] In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand (especially TRAIL (SEQ ID NO:66)), which involves administering to a cell which expresses a TR4 polypeptide, an effective amount of an antibody of the invention, preferably an antagonistic anti-TR4 antibody, capable of decreasing TR4 mediated signaling. Preferably, TR4 mediated signaling is decreased to treat a disease wherein increased apoptosis or NFκB expression is exhibited.

[0312] By TR4 "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis mediated by TRAIL receptor. By TR4 "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis mediated by TRAIL receptor. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit, respectively, apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

[0313] The antibodies of the invention can be used to treat, ameliorate or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of TR4 or TR4 ligand, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant TR4 expression and/or activity or aberrant TR4 ligand expression and/or activity includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0314] Further, antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which activate TRAIL receptor-mediated biological activities (e.g., the induction of apoptosis in TRAIL receptor expressing cells) can be administered to an animal to treat, prevent or ameliorate a disease or disorder described herein, particularly cancers and other hyperproliferative disorders. These antibodies may potentiate or activate either all or a subset of the

biological activities of TRAIL receptor, for example, by inducing a conformational change in TRAIL receptor. In a specific embodiment, an antibody of the present invention that increases TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the antibody is administered to an animal to treat, prevent or ameliorate a disease or disorder. In another embodiment, a combination of antibodies, a combination of antibody fragments, a combination of antibody variants, or a combination of antibodies, antibody fragments and/or antibody variants that increase TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the said antibodies or antibody fragments and/or antibody variants, is administered to an animal to treat, prevent or ameliorate a disease or disorder.

[0315] Further, antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which activate TR4-mediated biological activities (e.g., the induction of apoptosis in TR4 expressing cells) can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. These antibodies may potentiate or activate either all or a subset of the biological activities of TRAIL receptor, for example, by inducing a conformational change in TRAIL receptor. In a specific embodiment, an antibody of the present invention that increases TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the antibody

is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. In another embodiment, a combination of antibodies, a combination of antibody fragments, a combination of antibody variants, or a combination of antibodies, antibody fragments and/or antibody variants that increase TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the said antibodies or antibody fragments and/or antibody variants, is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression or lack of TR4 function or aberrant TR4 ligand expression or lack of TR4 ligand function.

**[0316]** Antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that function as agonists or antagonists of a TRAIL receptor, preferably of TR4 signal transduction, can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. For example, antibodies of the invention which mimic the action of TRAIL binding to TR4, in full or in part, TR4 agonists, may be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. As an alternative example, antibodies of the invention which disrupt or prevent the interaction between TR4 and its ligand or inhibit, reduce, or prevent signal transduction through TR4, may be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. Antibodies of the invention which do not prevent TR4 from binding its ligand but inhibit or downregulate TR4 signal transduction can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. The ability of an antibody of the invention to enhance, inhibit, upregulate or downregulate TR4 signal



transduction may be determined by techniques described herein or otherwise known in the art. For example, TRAIL-induced receptor activation and the activation of signaling molecules can be determined by detecting the association of adaptor proteins such as FADD and TRADD with TR4, by immunoprecipitation followed by western blot analysis (for example, as described herein).

[0317] Further, antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which activate TR4-mediated biological activities (e.g., the induction of apoptosis in TR4 expressing cells) can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. These antibodies may potentiate or activate either all or a subset of the biological activities of TRAIL receptor, for example, by inducing a conformational change in TRAIL receptor. In a specific embodiment, an antibody of the present invention that increases TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the antibody is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, lack of TR4 function, aberrant TR4 ligand expression, or lack of TR4 ligand function. In another embodiment, a combination of antibodies, a combination of antibody fragments, a combination of antibody variants, or a combination of antibodies, antibody fragments and/or antibody variants that increase TR4 activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least two-fold, at least three-fold, at least four fold, at least five fold, at least ten-fold, at least twenty-fold, at least fifty-fold, or at least one hundred-fold relative to TR4 activity in absence of the said antibodies or antibody fragments and/or antibody variants is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression or lack of TR4 function or aberrant TR4 ligand expression or lack of TR4 ligand function.

[0318] In a specific embodiment, an antibody of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that inhibits or downregulates, in full or in part, TR4 activity (e.g., stimulation of apoptosis) by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to TR4 activity in absence of the antibody is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, excessive TR4 function, aberrant TR4 ligand expression, or excessive TR4 ligand function. In another embodiment, a combination of antibodies, a combination of antibody fragments, a combination of antibody variants, or a combination of antibodies, antibody fragments, and/or variants that inhibit or downregulate TR4 activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to TR4 activity in absence of said antibodies, antibody fragments, and/or antibody variants, are administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant TR4 expression, excessive TR4 function, aberrant TR4 ligand expression, or excessive TR4 ligand function.

[0319] In one embodiment, therapeutic or pharmaceutical compositions of the invention are administered to an animal to treat, prevent or ameliorate a disease or disorder associated with increased apoptosis including, but not limited to, AIDS, neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration), myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In another embodiment, therapeutic or pharmaceutical compositions of the invention are administered to an animal to treat, prevent or ameliorate bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome.

[0320] Therapeutic or pharmaceutical compositions of the invention, may also be administered to treat, prevent, or ameliorate organ rejection or graft-versus-host disease (GVHD) and/or conditions associated therewith. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an

immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Cellular death induced by immune cell effector functions is apoptotic death. Thus, the administration of antibodies of the invention, (e.g., those that inhibit apoptosis), may be an effective therapy in preventing organ rejection or GVHD.

**[0321]** In another embodiment, therapeutic or pharmaceutical compositions of the invention are administered to an animal to treat, prevent or ameliorate infectious diseases. Infectious diseases include diseases associated with yeast, fungal, viral and bacterial infections. Viruses associated with viral infections which can be treated or prevented in accordance with this invention include, but are not limited to, retroviruses (e.g., human T-cell lymphotropic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (e.g., herpes simplex virus (HSV) types I and II, Epstein-Barr virus, HHV6-HHV8, and cytomegalovirus), arenaviruses (e.g., lassa fever virus), paramyxoviruses (e.g., morbillivirus virus, human respiratory syncytial virus, mumps, and pneumovirus), adenoviruses, bunyaviruses (e.g., hantavirus), coronaviruses, filoviruses (e.g., Ebola virus), flaviviruses (e.g., hepatitis C virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (e.g., hepatitis B viruses (HBV)), orthomyoviruses (e.g., influenza viruses A, B and C), papovaviruses (e.g., papillomaviruses), picornaviruses (e.g., rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (e.g., rotaviruses), togaviruses (e.g., rubella virus), rhabdoviruses (e.g., rabies virus). Microbial pathogens associated with bacterial infections include, but are not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter* (Vibrio) *fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Treponema pallidum*, *Treponema pertenuis*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*,

*Brucella suis*, *Brucella melitensis*, *Mycoplasma spp.*, *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia spp.*, and *Helicobacter pylori*.

[0322] In another embodiment, antibodies and antibody compositions of the present invention are used to treat, prevent, or ameliorate diseases associated with increased apoptosis including, but not limited to, AIDS, neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration), brain tumor or prion associated disease); autoimmune disorders (such as, multiple sclerosis, Rheumatoid Arthritis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In preferred embodiments, anti-TR4 antagonistic antibodies, prevent TRAIL from binding to the TRAIL receptors to which the antibodies are bound, but do not transduce the biological signal that results in apoptosis) are used to treat the diseases and disorders listed above.

[0323] Many of the pathologies associated with HIV are mediated by apoptosis, including HIV-induced nephropathy and HIV encephalitis. Thus, in additional preferred embodiments, antibodies, preferably antagonistic anti-TR4 antibodies, of the invention are used to treat AIDS and pathologies associated with AIDS. Another embodiment of the present invention is directed to the use of antibodies of the invention to reduce TRAIL-mediated death of T cells in HIV-infected patients.

[0324] In additional embodiments, antibodies of the present invention, particularly antagonistic anti-TR4 antibodies, are administered in combination with other inhibitors of T cell apoptosis. For example, Fas-mediated apoptosis has been implicated in loss of T cells in HIV individuals (Katsikis et al., J. Exp. Med. 181:2029-2036, 1995). Thus, a patient susceptible to both Fas ligand mediated and TRAIL mediated T cell death may be treated with both an agent that blocks TRAIL/TR4 interactions and an agent that blocks Fas-ligand/Fas interactions. Suitable agents for blocking binding of Fas-ligand to Fas include, but are not limited to, soluble Fas polypeptides; multimeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies that bind Fas without

transducing the biological signal that results in apoptosis; anti-Fas-ligand antibodies that block binding of Fas-ligand to Fas; and muteins of Fas-ligand that bind Fas but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method are monoclonal antibodies. Examples of suitable agents for blocking Fas-ligand/Fas interactions, including blocking anti-Fas monoclonal antibodies, are described in International application publication number WO 95/10540, hereby incorporated by reference.

**[0325]** Suitable agents, which also block binding of TRAIL to a TR4 that may be administered with the antibodies of the present invention include, but are not limited to, soluble TR4 polypeptides (e.g., a soluble form of OPG, TR5 (International application publication number WO 98/30693); a soluble form of TR4 (International publication number WO 98/32856); TR7/DR5 (International application publication number WO 98/41629); and TR10 (International application publication number WO 98/54202)); multimeric forms of soluble TR4 polypeptides; and TR4 antibodies that bind the TR4 without transducing the biological signal that results in apoptosis, anti-TRAIL antibodies that block binding of TRAIL to one or more TRAIL receptors, and muteins of TRAIL that bind TRAIL receptors but do not transduce the biological signal that results in apoptosis.

**[0326]** In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more than allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Antibodies of the present invention (e.g., agonistic antibodies of the invention) are able to suppress the immune response to both allografts and xenografts because lymphocytes activated and differentiated into effector cells will express the TR4 polypeptides, and thereby are susceptible to compounds which enhance apoptosis. Thus, the present invention further provides a method for creating immune privileged tissues. Antagonist of the invention can further be used in the treatment of Inflammatory Bowel-Disease.

[0327] Antibodies and antibody compositions of the invention may be useful for treating inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

[0328] In addition, due to lymphoblast expression of TR4 polypeptides, antibodies and antibody compositions of the invention may be used to treat this form of cancer. Further, antibodies and antibody compositions of the invention may be used to treat various chronic and acute forms of inflammation such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

[0329] In one embodiment, antibodies and antibody compositions of the invention may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

[0330] Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

[0331] Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

[0332] Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block,

long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

[0333] Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

[0334] Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

[0335] Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

[0336] Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

[0337] Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

[0338] Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

[0339] Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

[0340] Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

[0341] Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

[0342] In one embodiment, antibodies and antibody compositions of the invention is used to treat thrombotic microangiopathies. One such disorder is thrombotic thrombocytopenic purpura (TTP) (Kwaan, H.C., *Semin. Hematol.* 24:71 (1987); Thompson *et al.*, *Blood* 80:1890 (1992)). Increasing TTP-associated mortality rates have been reported by the U.S. Centers for Disease Control (Torok *et al.*, *Am. J. Hematol.* 50:84 (1995)). Plasma from patients afflicted with TTP (including HIV+ and HIV- patients) induces apoptosis of human endothelial cells of dermal microvascular origin, but not large vessel origin (Laurence *et al.*, *Blood* 87:3245 (1996)). Plasma of TTP patients thus is



thought to contain one or more factors that directly or indirectly induce apoptosis. As described in International patent application number WO 97/01715 (hereby incorporated by reference), TRAIL is present in the serum of TTP patients, and is likely to play a role in inducing apoptosis of microvascular endothelial cells. Another thrombotic microangiopathy is hemolytic-uremic syndrome (HUS) (Moake, J.L., *Lancet*, 343:393 (1994); Melnyk et al., (*Arch. Intern. Med.*, 155:2077 (1995); Thompson et al., *supra*). Thus, in one embodiment, the invention is directed to use of antibodies and antibody compositions of the invention to treat the condition that is often referred to as "adult HUS" (even though it can strike children as well). A disorder known as childhood/diarrhea-associated HUS differs in etiology from adult HUS. In another embodiment, conditions characterized by clotting of small blood vessels may be treated using of antibodies and antibody compositions of the invention. Such conditions include, but are not limited to, those described herein. For example, cardiac problems seen in about 5-10% of pediatric AIDS patients are believed to involve clotting of small blood vessels. Breakdown of the microvasculature in the heart has been reported in multiple sclerosis patients. As a further example, treatment of systemic lupus erythematosus (SLE) is contemplated. In one embodiment, antibodies and antibody compositions of the invention, preferably antagonistic anti-TR4 antibodies of the invention, may be administered *in vivo* to a patient afflicted with a thrombotic microangiopathy. Thus, the present invention provides a method for treating a thrombotic microangiopathy, involving use of an effective amount of an antibody or antibody composition of the invention.

[0343] Antibodies and antibody compositions of the invention may be employed in combination with other agents useful in treating a particular disorder. For example, in an *in vitro* study reported by Laurence et al. (*Blood* 87:3245 (1996)), some reduction of TTP plasma-mediated apoptosis of microvascular endothelial cells was achieved by using an anti-Fas blocking antibody, aurintricarboxylic acid, or normal plasma depleted of cryoprecipitate. Thus, a patient may be treated with an antibody or antibody composition of the invention in combination with an agent that inhibits Fas-ligand-mediated apoptosis of endothelial cells, such as, for example, an agent described above. In one embodiment, antibodies of the invention and an anti-FAS blocking antibody are both administered to a patient afflicted with a disorder characterized by thrombotic microangiopathy, such as TTP or HUS. Examples of blocking monoclonal antibodies directed against Fas antigen

(CD95) are described in International patent application publication number WO 95/10540, hereby incorporated by reference.

[0344] The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate (*Rastinejad et al., Cell* 56:345-355 (1989)). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al., Biotech.* 9:710-714 (1991); Folkman *et al., N. Engl. J. Med.*, 353:1757-1771 (1995); Auerbach *et al., J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al., Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

[0345] The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of an antibody or antibody compositions of the invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides of the invention include, but are not limited to those malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al., Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).

[0346] Additionally, ocular disorders associated with neovascularization which can be treated with an antibody or antibody composition of the invention include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity, macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases

associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

[0347] Additionally, disorders which can be treated with an antibody or antibody composition of the invention include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

[0348] Antibodies and antibody compositions of the invention are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), *Helicobacter pylori* infection, invasive Staphylococchia, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.))), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, autoimmune lymphoproliferative syndrome (ALPS), immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

[0349] Antibodies and antibody compositions of the invention are useful in promoting angiogenesis, wound healing (e.g., wounds, burns, and bone fractures).

[0350] Antibodies and antibody compositions of the invention are also useful as an adjuvant to enhance immune responsiveness to specific antigen, such as in anti-viral immune responses.

[0351] More generally, antibodies and antibody compositions of the invention are useful in regulating (i.e., elevating or reducing) immune response. For example, antibodies and antibody compositions of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and immunocompromised individuals. Alternatively, antibodies and antibody compositions of the invention are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, antibodies and antibody compositions of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

#### **Therapeutic/Prophylactic Compositions and Administration**

[0352] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of antibody (or fragment or variant thereof) or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, an antibody or fragment or variant thereof is substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to, animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably a human.

[0353] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0354] Various delivery systems are known and can be used to administer antibody or fragment or variant thereof of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem.

262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0355] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0356] In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1535 (1990); Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 3 17-327; see generally *ibid.*).

[0357] In yet another embodiment, the composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:20 1 (1987); Buchwald *et al.*, *Surgery* 88:507 (1980); Saudek *et al.*, *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design*

and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:71 (1983); see also Levy *et al.*, Science 228:190 (1985); During *et al.*, Ann. Neurol. 25:35 1 (1989); Howard *et al.*, J.Neurosurg. 7 1:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0358] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1535 (1990)).

[0359] In a specific embodiment where the composition of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see *e.g.*, Joliot *et al.*, Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0360] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of an antibody or a fragment thereof, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose,

sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

**[0361]** In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[0362]** The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0363] The amount of the composition of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0364] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of therapeutic or pharmaceutical compositions of the invention may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0365] Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments, or variants, (*e.g.*, derivatives), or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0366] It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to TR4, or polynucleotides encoding antibodies that immunospecifically bind to TR4, for both immunoassays and therapy of disorders related to TR4 polynucleotides or polypeptides, including fragments thereof. Such antibodies will preferably have an affinity for TR4 and/or TR4 polypeptide fragments. Preferred binding affinities include those with a dissociation constant or  $K_D$  of less than or equal to  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M, or  $10^{-5}$  M. More preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with a dissociation



constant or  $K_D$  less than or equal to  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M, or  $10^{-8}$  M. Even more preferably, antibodies of the invention bind TR4 polypeptides or fragments or variants thereof with a dissociation constant or  $K_D$  less than or equal to  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. In a preferred embodiment, antibodies of the invention induce apoptosis of TR4 expressing cells.

[0367] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0368] The antibody and antibody compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to chemotherapeutic agents, antibiotics, antivirals, anti-retroviral agents, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

#### **Combination Therapies with anti-TR4 antibodies, TRAIL, and/or Chemotherapeutic agents**

[0369] Anti-TR4 antibodies may be administered in combination with other anti-TR4 antibodies, TRAIL, and/or chemotherapeutics.

[0370] In specific embodiments, an antibody of the invention that specifically binds TR4 is used or administered in combination with a second antibody that specifically binds

TR7. In another embodiment, the antibodies specific for TR4 and TR7 are agonistic antibodies that induce apoptosis of TR4 expressing cells (e.g., cells that express TR4 and TR7). In a specific embodiment, the combination of anti-TR4 treatment and anti-TR7 treatment induces more apoptosis of TR4 and TR7 expressing cells than either anti-TR4 antibody treatment or anti-TR7 antibody treatment alone. The anti-TR4 and anti-TR7 antibodies can be administered either simultaneously, sequentially, or a combination of simultaneous or sequential administration throughout the dosage regimen. In another specific embodiment anti-TR4 and anti-TR7 antibodies are used or administered in combination with a chemotherapeutic drug, such as those described herein (see, for example, below and Example 4). In a particular embodiment, the synergistic induction of apoptosis resulting from anti-TR4 and anti-TR7 antibody treatment, is more evident or more pronounced when the anti-TR4 and anti-TR7 antibodies are used or administered in combination with a chemotherapeutic agent and/or a cross-linking reagent.

**[0371]** In a preferred embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin (adriamycin), bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, etoposide, Topotecan, 5-Fluorouracil, paclitaxel (Taxol), Cisplatin, Cytarabine, and IFN-gamma, irinotecan (Camptosar, CPT-11), irinotecan analogs, and gemcitabine (GEMZAR™)).

**[0372]** In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another

embodiment, antibody and antibody compositions of the invention are administered in combination with Rituximab. In a further embodiment, antibody and antibody compositions of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

**[0373]** In additional preferred embodiments, the compositions of the invention are administered in combination with TRAIL polypeptides or fragments or variants thereof, particularly of the extracellular soluble domain of TRAIL.

**[0374]** In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family or antibodies specific for TNF receptor family members. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/35904), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

### **Additional Combination Therapies**

**[0375]** In a more preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with anti-TNF antibody. In another

embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the antibody and antibody compositions of the invention are administered in combination ENBREL™. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specific embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specific embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), suflasalazine, anti-TNF antibody, and methotrexate.

**[0376]** The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may be administered alone or in combination with other therapeutic or prophylactic regimens (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, anti-angiogenesis and anti-inflammatory agents). Such combinatorial therapy may be administered sequentially and/or concomitantly.

**[0377]** Conventional nonspecific immunosuppressive agents, that may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

**[0378]** In specific embodiments, antibody and antibody compositions of the invention are administered in combination with immunosuppressants. Immunosuppressants

preparations that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

**[0379]** In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the antibody and antibody compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the antibody and antibody compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the antibody and antibody compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the antibody and antibody compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the antibody and antibody compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV.

**[0380]** The invention also encompasses combining the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) with other proposed or conventional hematopoietic therapies. Thus, for example, the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) can be combined with compounds that singly exhibit erythropoietic stimulatory effects, such as erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, and triiodothyronine. Also encompassed are combinations of the antibody and antibody compositions of the invention with compounds generally used to treat aplastic anemia, such as, for example,

methenolene, stanozolol, and nandrolone; to treat iron-deficiency anemia, such as, for example, iron preparations; to treat malignant anemia, such as, for example, vitamin B<sub>12</sub> and/or folic acid; and to treat hemolytic anemia, such as, for example, adrenocortical steroids, e.g., corticoids. See e.g., Resegotti et al., *Panminerva Medica*, 23:243-248 (1981); Kurtz, *FEBS Letters*, 14a:105-108 (1982); McGonigle et al., *Kidney Int.*, 25:437-444 (1984); and Pavlovic-Kantera, *Expt. Hematol.*, 8(supp. 8) 283-291 (1980), the contents of each of which are hereby incorporated by reference in their entireties.

**[0381]** Compounds that enhance the effects of or synergize with erythropoietin are also useful as adjuvants herein, and include but are not limited to, adrenergic agonists, thyroid hormones, androgens, hepatic erythropoietic factors, erythropoietins, and erythrocytogens, See for e.g., Dunn, "Current Concepts in Erythropoiesis", John Wiley and Sons (Chichester, England, 1983); Kalmani, *Kidney Int.*, 22:383-391 (1982); Shahidi, *New Eng. J. Med.*, 289:72-80 (1973); Urabe et al., *J. Exp. Med.*, 149:1314-1325 (1979); Billat et al., *Expt. Hematol.*, 10:135-140 (1982); Naughton et al., *Acta Haemat.*, 69:171-179 (1983); Cognote et al. in abstract 364, *Proceedings 7th Intl. Cong. of Endocrinology* (Quebec City, Quebec, July 1-7, 1984); and Rothman et al., 1982, *J. Surg. Oncol.*, 20:105-108 (1982). Methods for stimulating hematopoiesis comprise administering a hematopoietically effective amount (i.e., an amount which effects the formation of blood cells) of a pharmaceutical composition containing polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) to a patient. The polynucleotides and/or polypeptides of the invention and/or agonists or antagonists thereof is administered to the patient by any suitable technique, including but not limited to, parenteral, sublingual, topical, intrapulmonary and intranasal, and those techniques further discussed herein. The pharmaceutical composition optionally contains one or more members of the group consisting of erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, triiodothyronine, methenolene, stanozolol, and nandrolone, iron preparations, vitamin B<sub>12</sub>, folic acid and/or adrenocortical steroids.

**[0382]** In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0383] In an additional embodiment, the antibody and antibody compositions of the invention are administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0384] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0385] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0386] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0387] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to,

platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., *Cancer Res.* 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., *J. Bio. Chem.* 267:17321-17326, 1992); Chymostatin (Tomkinson et al., *Biochem J.* 286:475-480, 1992); Cyclodextrin Tetradasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., *Nature* 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, *J. Clin. Invest.* 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., *J. Biol. Chem.* 262(4):1659-1664, 1987); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., *Agents Actions* 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

**[0388]** Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC359555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3540) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

**[0389]** Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of



angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, AG-3540 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

**[0390]** In particular embodiments, the use of antibody and antibody compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of cancers and other hyperproliferative disorders.

**[0391]** In a further embodiment, the antibody and antibody compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

**[0392]** In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase

inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0393] In a further embodiment, the antibody and antibody compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0394] In other embodiments, antibody and antibody compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the antibody and antibody compositions of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and

LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, antibody and antibody compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0395] In an additional embodiment, the antibody and antibody compositions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, glucocorticoids and the

nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

**[0396]** The antibodies and antibody compositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with alum. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Viroosomal adjuvant technology. Vaccines that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

**[0397]** In another specific embodiment, antibody and antibody compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated therewith. In

one embodiment, antibody and antibody compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, antibody and antibody compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, antibody and antibody compositions of the invention are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, antibody and antibody compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0398] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0399] In another embodiment, antibody and antibody compositions of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with warfarin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin and aspirin.

[0400] In another embodiment, antibody and antibody compositions of the invention are administered in combination with an agent that suppresses the production of

anticardiolipin antibodies. In specific embodiments, the polynucleotides of the invention are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

**[0401]** In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

**[0402]** In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an NSAID.

**[0403]** In a nonexclusive embodiment, the antibody and antibody compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecaseprin (Chiron), T-714 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-671 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

**[0404]** In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California) and prednisolone.

**[0405]** In an additional embodiment, antibody and antibody compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the antibody and antibody compositions of the invention include, but

not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0406] CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0407] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In preferred embodiments, antibody and antibody compositions of the invention are administered with TRAIL receptor. In another embodiment, antibody and antibody compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the antibody and antibody compositions of the invention are administered in combination with IL4 and IL10.

[0408] In one embodiment, the antibody and antibody compositions of the invention are administered in combination with one or more chemokines. In specific embodiments, the antibody and antibody compositions of the invention are administered in combination with an  $\alpha$ (CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 ( $\gamma$ IP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a  $\beta$ (CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1 $\alpha$ ), macrophage inflammatory protein-1 beta (MIP-1 $\beta$ ), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte

chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1 $\gamma$ ), macrophage inflammatory protein-3 alpha (MIP-3 $\alpha$ ), macrophage inflammatory protein-3 beta (MIP-3 $\beta$ ), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the  $\gamma$ (C) chemokine, lymphotactin.

[0409] In another embodiment, the antibody and antibody compositions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the antibody and antibody compositions of the invention are administered with chemokine beta-8.

[0410] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the antibody and antibody compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0411] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

### **Demonstration of Therapeutic or Prophylactic Utility of a Composition**

[0412] The compounds of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific antibody or composition of the present invention is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered an antibody or composition of the present invention, and the effect of such an antibody or composition of the present invention upon the tissue sample is observed. In various specific embodiments, *in vitro* assays can be carried out with representative



cells of cell types involved in a patient's disorder, to determine if an antibody or composition of the present invention has a desired effect upon such cell types. Preferably, the antibodies or compositions of the invention are also tested in *in vitro* assays and animal model systems prior to administration to humans.

[0413] Antibodies or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, chicken, cows, monkeys, and rabbits. For *in vivo* testing of an antibody or composition's toxicity any animal model system known in the art may be used.

[0414] Antibodies or compositions of the invention can be tested for their ability to reduce tumor formation in *in vitro*, *ex vivo* and *in vivo* assays. Antibodies or compositions of the invention can also be tested for their ability to inhibit viral replication or reduce viral load in *in vitro* and *in vivo* assays. Antibodies or compositions of the invention can also be tested for their ability to reduce bacterial numbers in *in vitro* and *in vivo* assays known to those of skill in the art. Antibodies or compositions of the invention can also be tested for their ability to alleviate one or more symptoms associated with cancer, an immune disorder (*e.g.*, an inflammatory disease), a neurological disorder or an infectious disease. Antibodies or compositions of the invention can also be tested for their ability to decrease the time course of the infectious disease. Further, antibodies or compositions of the invention can be tested for their ability to increase the survival period of animals suffering from disease or disorder, including cancer, an immune disorder or an infectious disease. Techniques known to those of skill in the art can be used to analyze the function of the antibodies or compositions of the invention *in vivo*.

[0415] Efficacy in treating or preventing viral infection may be demonstrated by detecting the ability of an antibody or composition of the invention to inhibit the replication of the virus, to inhibit transmission or prevent the virus from establishing itself in its host, or to prevent, ameliorate or alleviate the symptoms of disease or a progression. The treatment is considered therapeutic if there is, for example, a reduction in viral load, amelioration of one or more symptoms, or a decrease in mortality and/or morbidity following administration of an antibody or composition of the invention.

[0416] Antibodies or compositions of the invention can be tested for their ability to modulate the biological activity of immune cells by contacting immune cells, preferably human immune cells (*e.g.*, T-cells, B-cells, and Natural Killer cells), with an antibody or composition of the invention or a control compound and determining the ability of the

antibody or composition of the invention to modulate (*i.e.*, increase or decrease) the biological activity of immune cells. The ability of an antibody or composition of the invention to modulate the biological activity of immune cells can be assessed by detecting the expression of antigens, detecting the proliferation of immune cells (*i.e.*, B-cell proliferation), detecting the activation of signaling molecules, detecting the effector function of immune cells, or detecting the differentiation of immune cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by <sup>3</sup>H-thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but not limited to, competitive and non-competitive assay systems using techniques such as western blots, immunohistochemistry radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs). In a preferred embodiment, the ability of an antibody or composition of the invention to induce B-cell proliferation is measured. In another preferred embodiment, the ability of an antibody or composition of the invention to modulate immunoglobulin expression is measured.

### **Panels/Mixtures**

[0417] The present invention also provides for mixtures of antibodies (including scFvs and other molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to TR4 or a fragment or variant thereof, wherein the mixture has at least one, two, three, four, five or more different antibodies of the invention. In specific embodiments, the invention provides mixtures of at least 2, preferably at least 4, at least 6, at least 8, at least 10, at least 12, at least 15, at least 20, or at least 25 different antibodies that immunospecifically bind to TR4 or fragments or variants thereof, wherein at least 1, at least 2, at least 4, at least 6, or at least 10, antibodies of the mixture is an antibody of the invention. In a specific embodiment, each antibody of the mixture is an antibody of the invention.

[0418] The present invention also provides for panels of antibodies (including scFvs and other molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to TR4 or a fragment or variant thereof, wherein the panel has at least one, two, three, four, five or more different antibodies of the invention. In specific embodiments, the invention provides for panels of antibodies that have different affinities for TRAIL receptor, different specificities for TRAIL receptor, or different dissociation rates. The invention provides panels of at least 10, preferably at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, or at least 1000, antibodies. Panels of antibodies can be used, for example, in 96 well plates for assays such as ELISAs.

[0419] The present invention further provides for compositions comprising, one or more antibodies (including molecules comprising, or alternatively consisting of antibody fragments or variants of the invention). In one embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH domains of a one or more of the scFvs referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR1s of a VH domain of one or more of the scFvs referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR2s of a VH domain of one or more of the scFvs referred to in Table 1, or a variant thereof. In a preferred embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR3s as of a VH domain of one or more of the scFvs referred to in Table 1, or a variant thereof.

[0420] Other embodiments of the present invention providing for compositions comprising, one or more antibodies (including molecules comprising, or alternatively consisting of antibody fragments or variants of the invention) are listed below. In another

embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL domains of one or more of the scFvs referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL CDR1s domains of one or more of the scFvs referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL CDR2s of one or more of the scFvs referred to in Table 1, or a variant thereof. In a preferred embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL CDR3s domains of one or more of the scFvs referred to in Table 1, or a variant thereof.

### **Kits**

**[0421]** The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

**[0422]** The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In an alternative embodiment, a kit comprises an antibody fragment that immunospecifically binds to TR4 polypeptides or fragments or variants thereof. In a specific embodiment, the kits of the present invention contain a substantially isolated TR4 polypeptide or fragment or variant thereof as a control. Preferably, the kits of the present invention further comprise a control antibody which does not react with any, some or all TRAIL receptors. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to TR4 polypeptides (*e.g.*, the antibody may be conjugated to a detectable substrate such

as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized TRAIL receptor. The TR4 provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which TR4 is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to TR4 can be detected by binding of the said reporter-labeled antibody.

**[0423]** In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with a TRAIL receptor, and means for detecting the binding of TR4 polypeptides to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

**[0424]** In one diagnostic configuration, test serum is reacted with a solid phase reagent having surface-bound TRAIL receptors obtained by the methods of the present invention. After TR4 polypeptides bind to a specific antibody, the unbound serum components are removed by washing, reporter-labeled anti-human antibody is added, unbound anti-human antibody is removed by washing, and a reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-TR4 antibody on the solid support. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate.

**[0425]** The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0426] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant TRAIL receptor, and a reporter-labeled anti-human antibody for detecting surface-bound anti-TR4 antibody.

### **Placental Expression of TRAIL Receptors**

[0427] The expression of tumor necrosis family receptors and ligands in whole placenta and in placental macrophage and trophoblast cell lines have been carefully examined. It has been shown that trophoblasts express TR7 and TR5 but not TR10 are entirely resistant to killing by recombinant TRAIL whereas macrophages, which express TR4, TR7 and TR10 but not TR5, are sensitive (Phillips et al., J. Immunol 15:6053-9 (1999) which is incorporated in its entirety by reference herein). Thus the methods for using anti-TR4 antibodies described herein, may also be used on placenta and placental cell types (e.g., macrophages and trophoblast cells) to prevent, treat, diagnose, ameliorate, or monitor diseases and disorders of the placenta placental cell types.

### **Gene Therapy**

[0428] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of TRAIL Receptors and/or its ligands (e.g., TRAIL), by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0429] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0430] For general reviews of the methods of gene therapy, see Goldspiel *et al.*, Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.),

Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[0431] In a preferred aspect, a composition of the invention comprises, or alternatively consists of, nucleic acids encoding an antibody, said nucleic acids being part of an expression vector that expresses the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra *et al.*, Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is an scFv; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments or variants thereof, of an antibody.

[0432] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[0433] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another

embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06 180; WO 92/22715; W092/203 16; W093/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra *et al.*, Nature 342:435-438 (1989)).

[0434] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention or fragments or variants thereof are used. For example, a retroviral vector can be used (see Miller *et al.*, Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen *et al.*, Biotherapy 6:29 1-302 (1994), which describes the use of a retroviral vector to deliver the *mdr 1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, J. Clin. Invest. 93:644-651(1994); Klein *et al.*, Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[0435] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, Science 252:431-434 (1991); Rosenfeld *et al.*, Cell 68:143- 155 (1992); Mastrangeli *et al.*, J. Clin. Invest.



91:225-234 (1993); PCT Publication W094/12649; and Wang, *et al.*, Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0436] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0437] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0438] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, Meth. Enzymol. 217:599-718 (1993); Cohen *et al.*, Meth. Enzymol. 217:718-644 (1993); Clin. Pharma. Ther. 29:69-92m (1985)) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0439] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0440] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular

hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0441] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0442] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or fragment thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO 94/08598; Stemple and Anderson, *Cell* 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.* 71:771 (1986)).

[0443] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

### **Examples**

#### **Example 1: Isolation and characterization of scFvs referred to in Table 1**

##### **Rescue of large scFv libraries**

[0444] An scFv library of up to  $1 \times 10^{11}$  clones, which is an expanded version of the  $1.38 \times 10^{10}$  library described (Vaughan *et. al.* (1996) *Nature Biotechnology* 14: 309-314), was used to select antibodies specific for TR4. Phage were rescued by taking  $3 \times 10^{10}$  cells from a glycerol stock culture and growing in 2YTAG (2YT media supplemented with 100 $\mu$ g/ml ampicillin and 2% (w/v) glucose) at 37°C for 2h with shaking. M13K07 helper phage (Stratagene) was added to the culture at a multiplicity of infection (moi) of approximately 10. The culture was incubated stationary at 37°C for 15 min followed by 45 min with light aeration (200 rpm) at the same temperature. The culture was centrifuged and the cells were resuspended in 500 ml 2YTAK (2YT media supplemented with 100  $\mu$ g/ml kanamycin), and the culture incubated overnight at 30°C with good aeration (300 rpm). Phage particles were purified and concentrated by three cycles of polyethylene glycol (PEG) precipitation (20% PEG 6000, 2.5M NaCl) on ice, then resuspended in

phosphate buffered saline (PBS) at  $10^{12}$  transducing units (tu)/ml, titrated as ampicillin resistant clones.

#### Panning of scFv libraries on TR4

[0445] Soluble, purified TR4 fusion protein was produced by HGS. Purified phagemids were first deselected on an irrelevant fusion protein to remove any irrelevant binders. To do this, 500µl of an irrelevant fusion protein was immobilised (10µg/ml in PBS) on a 75mm x 12mm immunotube (Nunc; Maxisorp) overnight at 4°C. After washing 3 times with PBS, the tube was filled with 3% MPBS (3% 'Marvel' skimmed milk powder in PBS) and blocked for 2 h at 37°C. The wash was repeated and phagemid particles ( $10^{13}$  tu) in 500µl 3% MPBS containing 100µg/ml irrelevant fusion protein were added and the tube incubated stationary at 37°C for 1 h. The phagemid particles were then transferred to an immunotube which had been coated with TR4 (10µg/ml in PBS) overnight at 4°C and blocked for 2 h at 37°C with 3% MPBS. The tube was incubated stationary at 37°C for 1 hour and then washed 10 times with PBST (PBS containing 0.1% (v/v) Tween 20), and 10 times with PBS. Bound phagemid particles were eluted with 1ml 100mM triethylamine for 10min at room temperature, then immediately neutralised with 0.5ml 1M Tris.HCl (pH 7.4). The eluted phage were used to infect 10 ml exponentially growing *E.coli* TG1. Infected cells were grown in 2YT broth for 1 h at 37°C with light aeration, then streaked onto 2YTAG agar plates (243mm x 243mm; Nunc) and incubated overnight at 30°C. Colonies were scraped off the plates into 10 ml of 2YT broth and 15% (v/v) glycerol added for storage -70°C.

[0446] Glycerol stock cultures from the first round of panning on TR4 fusion protein were then superinfected with helper phage and rescued to give phagemid particles for the second round of panning. 25 µl of glycerol stock was inoculated into 25 ml 2TYAG broth, and incubated at 37°C with good aeration until the OD<sub>600nm</sub> reached 0.7. M13K07 helper phage (moi =10) was added to the culture which was then incubated stationary for 15 min at 37°C then with shaking for 45 min at the same temperature. The culture was centrifuged, the cells were resuspended in 50 ml prewarmed 2YTAK and rescue was performed overnight at 30°C as before. Phagemid particles were purified and concentrated as before and resuspended in PBS to  $10^{13}$  tu/ml. Repertoires harvested at subsequent rounds of selection were superinfected and rescued in the same way.

[0447] Four rounds of panning selection were performed and individual colonies screened by phage ELISA for binding to TR4.

#### **Phage ELISA**

[0448] To determine the specificity of each of the antibodies, a phage ELISA was performed for each antibody against TR4 fusion protein and an irrelevant fusion protein.

[0449] Individual *E. coli* colonies containing phagemid were inoculated into 96 well plates containing 100 µl 2TYAG medium per well. Plates were incubated 37°C for 4 hours, shaking. M13K07 helper phage was added to each well to an moi of 10 and the plates were incubated for a further 1 hour at 37°C. The plates were centrifuged in a benchtop centrifuge at 2000 rpm for 10 minutes. The supernatant was removed and cell pellets were resuspended in 100 µl 2TYAK and incubated at 30°C overnight, shaking. The next day, plates were centrifuged at 2000 rpm for 10 min and 100 µl phage-containing supernatant from each well carefully transferred into a fresh 96-well plate. Twenty µl of 6xMPBS was added to each well, and incubated at room temperature for 1 hour to block the phage prior to ELISA.

[0450] Flexible 96-well plates (Falcon) were coated overnight at 4°C with human TR4 (1 µg/ml) or an irrelevant fusion protein (1 µg/ml). Both antigens were coated in PBS. After coating, the solutions were removed from the wells, and the plates were blocked for 1 hour at room temperature in MPBS. The plates were washed 3 times with PBS and then 50 µl of preblocked phage was added to each well. The plates were incubated at room temperature for 1 hour and then washed with 3 changes of PBST followed by 3 changes of PBS.

[0451] To each well, 50 µl of an anti-M13–HRP conjugate (Pharmacia) at a 1 in 5000 dilution in MPBS was added and the plates incubated at room temperature for 1 hour. Each plate was washed three times with PBST followed by three times with PBS.

[0452] Fifty µl of TMB substrate was then added to each well, and incubated at room temperature for 30 minutes or until colour development. The reaction was stopped by the addition of 25 µl of 0.5 M H<sub>2</sub>SO<sub>4</sub>. The signal generated was measured by reading the absorbance at 450nm (A<sub>450</sub>) using a microtitre plate reader (Bio-Rad 3550).

[0453] From a panel of 1500 clones which were screened by ELISA, 250 antibodies were identified which bound TR4 fusion protein but not an irrelevant fusion protein. The

results of a typical plate of clones are shown in Figure 1. ninety-five percent of isolated antibodies recognised TR4 fusion protein but not an irrelevant fusion protein.

#### Specificity Phage ELISA

[0454] To determine the specificity of the antibodies which bound TR4, a phage ELISA was performed against human TR4 fusion protein, and a panel of related and unrelated human antigens TR7, TR5, TR10, BlyS (described in International Patent Publication Numbers WO98/18921 and WO00/50597, which are both herein incorporated by reference in their entireties), irrelevant fusion protein and BSA.

[0455] Individual *E.coli* colonies containing phagemid were inoculated into 5ml 2YTAG and incubated at 37°C for 4 hours, shaking. M13K07 helper phage (Pharmacia) was added to each tube to an moi of 10 and incubated for 30 min at 37°C for 1 hour, the first 30 minutes static and the final 30 minutes with gentle shaking. Cells were pelleted by centrifugation at 3,500 rpm for 10 minutes. The phage containing supernatant (5 ml) was carefully transferred to a fresh tube, 1ml of 6MPBS added and then incubated at room temperature for 1 hour to pre-block the phage prior to ELISA.

[0456] Flexible 96-well plates (Falcon) were coated overnight at 4°C with each antigen (1 µg/ml). All antigens were coated in PBS. After coating, the solutions were removed from the wells, and the plates were blocked for 1 hour at room temperature in MPBS. The plates were washed 3 times with PBS and then 50 µl of pre-blocked phage was added to each well. The plates were incubated at room temperature for 1 hour and then washed with 3 changes of PBST followed by 3 changes of PBS.

[0457] To each well, 50 µl of an anti-M13-HRP conjugate (Pharmacia) at a 1 in 5000 dilution in MPBS was added and the plates incubated at room temperature for 1 hour. Each plate was washed three times with PBST followed by three times with PBS.

[0458] Fifty µl of TMB substrate was then added to each well, and incubated at room temperature for 30 minutes or until colour development. The reaction was stopped by the addition of 25 µl of 0.5 M H<sub>2</sub>SO<sub>4</sub>. The signal generated was measured by reading the absorbance at 450nm (A<sub>450</sub>) using a microtitre plate reader (Bio-Rad 3550).

[0459] Using this assay, scFVs T1014F08, T1014G03, T1014A04, T1014G04, T1014B11, T1017D09 were shown to bind TR4 but not TR7, TR5, TR10, BlyS, or an irrelevant fusion protein, indicating that the antibodies specifically recognise TR4.

**Example 2:****Biacore Analysis of the Affinity of TR4 Binding Polypeptides****Materials**

BIAcore 2000 instrument  
BIAcore 2000 control software, version 3.1.1  
BIAevaluation, version 3.1  
BIAcore CM5 Sensor Chip, Cat # BR-1000-14 Lot# 0364 (BIAcore)  
HBS-EP Buffer  
Amine Coupling Kit Cat# BR-1000-50 (BIAcore)  
EDC, #1048-950345(BIAcore)  
NHS, #1048-950345(BIAcore)  
Ethanolamine, #1048-950345(BIAcore)  
  
10mM Acetate, pH 4.0 Cat# BR1003-50 Lot#1821-9503844(BIAcore)  
TRAIL-FLAG (Alexis Biochemicals Cat# 522-003-C010 #L04793/a)  
The temperature was 25°C for all experiments.

**General Methods**

[0460] TR4, TR5, TR7 and TR10 (in the form of Fc fusion proteins) are immobilized on individual flow cells of a BIAcore sensor chip. The TR4-Fc fusion protein comprises residues M1-I240 of TR4 (SEQ ID NO:1). Post translational processing of this fusion protein results in a TR4-Fc fusion protein that comprises residues A109-I240 of TR4 (SEQ ID NO:1). The TR5-Fc fusion protein comprises residues R70-S282 of TR5 (SEQ ID NO:2). This protein is expressed in a baculovirus expression system that utilizes the GP signal peptide. Thus, post-translational processing of this fusion protein results in a TR5-Fc fusion protein that comprises the last 3 residues of the GP signal peptide (Ala-Asp-Pro) fused to R70-S282 of TR5 (SEQ ID NO:2) fused to the Fc region. The TR7-Fc fusion protein comprises residues E52-G184 of TR7 (SEQ ID NO:3). This protein is expressed in a baculovirus expression system that utilizes the GP signal peptide. Thus, post-translational processing of this fusion protein results in a TR7-Fc fusion protein that comprises the last 3 residues of the GP signal peptide (Ala-Asp-Pro) fused to E52-G184 of TR5 (SEQ ID NO:3) fused to the Fc region. The TR10-Fc fusion protein comprises residues M1-G204 of TR10 (SEQ ID NO:4). Post translational processing of this fusion protein results in a TR10-Fc fusion protein that comprises residues A56-G204 of TR10 (SEQ ID NO:4).

[0461] Amine coupling is used to covalently bind each receptor (Fc) to the dextran matrix on the CM5 sensor chip. The optimal pH for this coupling is analyzed using preconcentration experiments ranging from pH 4-7 and is determined based on the slope of the binding.

[0462] The actual coupling is performed using the manual injection mode. A target level of ~2000RU is set as the goal for all flow cells. (This may vary from 2000-3100 depending on the molecular weight of the receptor). The concentration of all receptors for immobilization was 10ug/ml in 10mM acetate, pH 4.0. The entire immobilization experiment is performed at 5microliters/min. Contact time for the EDC/NHS injection is 7 minutes. The ethanolamine is injected for 7 minutes.

[0463] The screening may be performed with the following procedures. The flow rate for the entire binding cycle is 25microliters/minute. Antibodies corresponding to scFvs are diluted in HBS-EP and flown through all four cells with immobilized TRAIL receptors. Each sample is in contact with the receptors for 4 minutes. Regeneration is performed using 15 microliters of 25mM NaOH. Successful regeneration is considered as not only removing the antibody, but also not denaturing the immobilized receptor.

[0464] The positive control for this screening experiment is an identical (in flow rate and length of time) injection of the soluble TRAIL ligand. The concentration is 1 microgram/mL. The negative control is a 1:10 dilution in HBS-EP of the antibody diluent. Data may be analyzed using the BIAevaluation software package.

#### **Biacore Analysis of Anti-TR4 Antibodies**

[0465] In the following experiment based on the general methods described above, the affinities of certain antibodies (corresponding to the scFvs of the invention) for TR4 were determined using a "double reference subtraction" method using TR4:Fc receptor in the experimental flow cell and TR2:Fc (comprising amino acids 1-240 of TR2 as disclosed in WO96/34095) as a negative control.

#### **Immobilization:**

[0466] The optimal pH for this coupling was analyzed using preconcentration experiments ranging from pH 4-7 and was determined to be pH 4.0 for both the TR4 and the TR2 receptor. Amine coupling was used to covalently bind each receptor (fc) to the dextran matrix on the CM5 sensor chip. The immobilization experiment was performed

using the manual injection mode. The entire immobilization experiment was performed at 5  $\mu\text{L}/\text{min}$ . A 3-minute injection of EDC/NHS (1:1) was applied to each flow cell to active esters. A target level of  $\sim 200\text{RU}$  was set as the goal for all each flow cell. Fc-fusion receptors, TR4 and TR2 at 5  $\mu\text{g}/\text{mL}$ , were immobilized onto individual flow cells of a sensor chip. The amount applied varied from 8-14  $\mu\text{L}$ . A 3-minute ethanolamine injection completed the immobilization experiment by inactivating the esters.

### Kinetics:

[0467] The kinetics cycles were performed as follows: The flow rate for the entire cycle was 25  $\mu\text{L}/\text{minute}$  with the flow path including both the control (TR2) and experimental (TR4) flow cell at all times. A 1-minute buffer injection was applied to stabilize the baseline. The purified antibody (IgG1 antibody comprising the VH and VL domains of each of the T1014A04, T1014G03, T1014F08, and T14G04 scFvs) was diluted from 10  $\mu\text{g}/\text{mL}$  (65 nM) to 0.115  $\mu\text{g}/\text{mL}$  (0.75 nM) in running buffer and tested in duplicate. Each concentration was in contact with the control (TR2) and the experimental (TR4) flow cell during a 4- minute association and a 10-minute dissociation phase. Regeneration was performed using 25 mM NaOH from 5-12  $\mu\text{L}$  depending on the sample concentration.

### Evaluation:

[0468] A double-reference subtraction was performed, which refers to the control flow cell subtraction for every cycle, in addition to a buffer cycle subtraction. The 1:1 Langmuir model was used for all evaluation fitting. Results of these experiments are shown in Table 5 below.

**Table 5: Affinity of antibodies for TR4**

Clone	$k_a$	$k_d$	$K_D$	$\text{Chi}^2$
T1014A04	$5.67 \times 10^5$	$2.65 \times 10^{-4}$	$4.68 \times 10^{-10}$	2
T1014G03	$3.50 \times 10^5$	$1.94 \times 10^{-4}$	$5.54 \times 10^{-10}$	0.76
T1014F08	$1.23 \times 10^6$	$1.02 \times 10^{-4}$	$8.27 \times 10^{-10}$	1.83
T1014G04	$6.05 \times 10^5$	$1.18 \times 10^{-4}$	$1.94 \times 10^{-10}$	1.14



**Example 3:****Inhibition of Binding of Biotinylated-TRAIL to TR4****I. Materials:**

10X PBS (Quality Biological Cat 130-069-161, Lot 708712)  
Immulon 4 microplate (Dynex Cat 3855, Lot ND540319)  
Bovine Serum Albumin fraction V (Sigma, #58H0456)  
Tri Hydroxy Methyl Amino Methane (TRIS BASE)  
Tween 20 (Sigma)  
Goat anti-human Fc (Sigma, I-2136, #89H4871)  
TR-4:Fc (as described above)  
Biotinylated TRAIL (AM100200-Peprotech)  
HRP-Streptavidin (Vector, #L0328)  
TMB Peroxidase Microwell Substrate System (KPL, Kirkegaard & Perry Laboratories, Inc.)  
H<sub>2</sub>SO<sub>4</sub> (Fisher)  
96 well dilution plate (Costar)

**II. Buffers:**

Coating buffer (1X PBS)  
Blocking buffer (3% BSA in PBS)  
All-purpose Diluent (1% BSA in PBST)  
Washing buffer (0.1% Tween 20 and 1x PBS)

**III. Methods**

**[0469]** Goat anti-human Fc is diluted to 0.1 micrograms/ml in coating buffer. An Immulon 4 microplate is coated with 100 microliters per well of the Goat anti-human Fc solution and incubated overnight at 4°C. The coating solution is decanted from the plate, and blocking solution is dispensed at 200 microliters per well. The plate is incubated at room temperature for 1 hour. After the 1 hour incubation period, the blocking solution is decanted from the plate and 1 microgram/mL of TR4-Fc is dispensed at 100 microliters/well and incubated for 2 hours at room temperature. After the incubation, the plate is washed five times manually using a Wheaton manifold.

**[0470]** Antibodies corresponding to scFvs of the present invention are (previously) prepared in a low binding dilution plate using diluent. The antibodies are prepared in duplicate and are diluted from the stock concentration with 2.5 fold dilutions for the 7 subsequent wells. If a purified form of the antibody is available, the starting concentration is 5 micrograms/mL. The positive control (TR4-Fc) is diluted from 5 micrograms/mL. 100 microliters is transferred into the ELISA plate and pre-incubated for 30 minutes at room temperature. 20 microliters of biotinylated TRAIL is added at 5 micrograms/mL to

the 100  $\mu$ L of the supernatant and mixed. The combined 120 microliters is incubated for 2 hours at RT.

[0471] After the two-hour incubation, the washing cycle is repeated and the plate decanted and blotted. HRP-streptavidin is diluted 1:2000 and 100 microliters per well is dispensed. Incubation is for one hour at room temperature. Meanwhile, equal amounts of the TMB peroxidase substrate and the peroxidase solution B are withdrawn and the solutions are equilibrated to room temperature.

[0472] After the one-hour incubation, the plate is decanted and washed with PBST five times and blotted. The TMB peroxidase substrate and the peroxidase solution B are combined and 100 microliters is dispensed to each well. The color developed at room temperature for 15 minutes. The color development is quenched by adding 50 microliters of the 1 M  $\text{H}_2\text{SO}_4$  to each well. The plate is immediately read at 450 nm using the spectrometer from Molecular Devices.

[0473] The IC-50, i.e., the concentration of purified antibody that resulted in 50% inhibition of plateau binding, is then measured. For comparison purposes, a TR4 polypeptide is used as a sample in this assay.

#### **Example 4:**

##### **Assay for Ability of Anti-TRAIL-R1 (TR4) Antibodies to induce Apoptosis**

##### **General Methods:**

[0474] Anti-TR4 antibodies are tested for their ability to induce apoptosis of TR4 expressing cells, alone or in combination with chemotherapeutic or cross-linking agents. Briefly, antibodies are tested for activity to induce TR4 mediated apoptosis of TR4 expressing cell lines, SW480 and HeLa. HT1080 fibrosarcoma cell line, which does not express TR4, is used as a negative control.

[0475] To induce apoptosis, either HeLa or SW480 cells are incubated with the indicated concentration of monoclonal antibodies or a human IgG2a control antibody. One day prior to assay, cells ( $0.3 \times 10^6$  cells/ml; 100  $\mu$ L/well) are seeded into wells of a 96-well plate and allowed to adhere overnight. The following day, the test antibody is added either in the presence or absence of 2.0 micrograms/ml cycloheximide (Sigma R75010-7). In some experiments, the potency of anti-TR4 monoclonal antibody is compared to rhuTRAIL-FLAG protein (Alexis Biochemicals). rhuTRAIL is used at the indicated concentrations in the presence of anti-FLAG enhancer antibody at 2 micrograms/ml. The

effect of secondary crosslinking is also assessed by measuring the ability of the monoclonal antibodies to kill cells alone, or in the presence of a secondary goat-anti-human Ig Fc specific antibody (SIGMA). The secondary crosslinking antibody is added to cells at an equivalent concentration as the test monoclonal antibody. The ability of a chemotherapeutic agent to sensitize cells to killing via the monoclonal antibody is assessed by treating either Hela or SW480 cells with monoclonal antibody in the presence of Topotecan (Hycamtin, SmithKline Beecham NDC 0007-4201-01).

[0476] Assays are performed for 16-18hrs at 37°C, after which viability is revealed using the reagent, Alamar Blue (Biosource, cat. # DAL1100) using conditions suggested by the manufacturer. Alamar Blue fluorescence is detected using the CytoFluor fluorescence reader at 530 nm excitation and 590 emission. Results are expressed as a percent viability compared to untreated cells.

[0477] Other chemotherapeutics that may be tested in this assay (and used in treatment regimens in conjunction with the antibodies of the present invention) include, for example, 5-Fluorouracil, Etoposide, Taxol, Cisplatin, Cytarabine (Cytosar), IFN gamma, camptothecin, irinotecan (camptosar, CPT-11), adriamycin (doxorubicin), methotrexate, paraplatinin, interferon-alpha, paclitaxel, docetaxel, the NF-kappa-B inhibitor SN50, and gemcitabine (Gemzar™). Other cell lines that may be tested in this assay include, for example, the human Burkitt lymphoma line ST486, human breast carcinoma cell line MDA-MB-231, the human uterine carcinoma cell line RL-95, the human lung carcinoma cell line SK-MES-1, human colon cancer cell lines, LS174T, HT29, and HCT116, the su.86.86 and CFPAC pancreatic cancer cell lines, the human ovarian cancer cell line TOV21G, and the human hepatocellular cancer cell line SNU449. Cancers of the tissues corresponding to the tissues from which these cancer cell lines were derived may be treated with the therapeutic compositions in accordance with the invention.

#### **Analysis of Anti-TR4 Antibodies**

[0478] Using the assay above, several scFVs of the present invention that had been converted to whole IgG1 molecules were tested for the ability to induce apoptosis of TR4 1 (TR4) expressing cells. The IgG1 format of T1014A04 induces apoptosis of SW480 cells in the presence of a cross linking agent, but in the absence of cycloheximide. In the presence of cycloheximide, but with or without a crosslinking reagent, the IgG1 format of T1014A04 induces apoptosis of SW480 and HeLa cells. Killing of SW480 and HeLa cells

by treatment with the IgG1 format of T1014A04 in the presence of cycloheximide is greater when crosslinking reagent is also used. In fact in the presence of crosslinking reagent, and cycloheximide, the IgG1 format of T1014A01 is able to induce more apoptosis than an equal concentration (in ng/ml) of soluble TRAIL. The IgG1 format of T1015A02 does not induce killing in the absence of the sensitizing agent cycloheximide. In the presence of cycloheximide, with or without a crosslinking reagent, the IgG1 format of T1015A02 induces apoptosis of SW480 cells but not HeLa cells. Killing of SW480 by treatment with the IgG1 format of T1015A02 is greater in the presence of a crosslinking reagent.

[0479] In addition, the assay described in this example may also be used to test the effect of more than one anti-TR4 antibody on TR4 expressing cells. For example, cells may be treated with both an antibody that specifically binds TR4 and an antibody that specifically binds TR7. As above, this experiment may be performed in the presence of absence of one or more chemotherapeutic agents or crosslinking agents. In another variation of the present experiment antibodies of the invention may be tested for the apoptosis inducing effect when used in the presence of TRAIL. The amount of apoptosis induced by dual treatment with anti-TR4 and anti-TR7 may be synergistic compared to treatment with either anti-TR4 or anti-TR7 alone. Such an effect may be more pronounced when the experiment is performed in the presence of chemotherapeutic and/or crosslinking agents.

### **Example 5:**

#### **Identification and Cloning of VH and VL domains**

[0480] One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville, MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and then centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at

4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75% ethanol. Following washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can be determined using optical density measurements.

[0481] cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 6. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes were stored 4°C.

**Table 6: Primer Sequences Used to Amplify VH and VL domains.**

<b>Primer name</b>	<b>SEQ ID NO</b>	<b>Primer Sequence (5'-3')</b>
<b>VH Primers</b>		
Hu VH1-5'	6	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	7	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	8	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	9	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	10	GAGGTGCAGCTGTTGCAGTCTGC
Hu VH6-5'	11	CAGGTACAGCTGCAGCAGTCAGG
Hu JH1,2-5'	12	TGAGGAGACGGTGACCAGGGTGCC
Hu JH3-5'	13	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	14	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	15	TGAGGAGACGGTGACCGTGGTCCC
<b>VL Primers</b>		
Hu Vkappa1-5'	16	GACATCCAGATGACCCAGTCTCC
Hu Vkappa2a-5'	17	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5'	18	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	19	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5'	20	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	21	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	22	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda1-5'	23	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	24	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	25	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5'	26	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5'	27	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	28	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5'	29	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	30	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3'	31	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3'	32	ACGTTTGATATCCACTTGGTCCC
Hu Jkappa4-3'	33	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa5-3'	34	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3'	35	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3'	36	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3-3'	37	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3'	38	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3'	39	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3'	40	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3'	41	AATTTTATGCTGACTCAGCCCCA

[0482] PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc. , Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of E.

coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

**Example 6: Anti-TR4 Antibodies Retard the Growth of Tumor Cells in Nude Mice.**

[0483] SW480 (colorectal adenocarcinoma) tumor cell line was maintained in vitro in Leibovitz's L-15 medium supplemented with fetal bovine serum, glutamine and antibiotics as per the instructions received from American Type Culture Collection. Cells at passage 3-10 were used for the in vivo studies. The tumor cells were harvested from the T-150 flasks, rinsed with sterile PBS and then resuspended in sterile saline at a density of 5(10<sup>4</sup>) cells/ul. Tumor cells were implanted subcutaneously on the upper back or flanks of Swiss athymic mice at a density of 10<sup>7</sup> cells per site, 2 sites per animal. In preventive (de novo) tumor models, chemotherapeutic agents and antibody treatments were initiated 24 hr post-tumor cell inoculation.

[0484] The antibody treatment, with an antibody comprising the VH and VL domain from either T1014A04 or T1014G03 (in this example hereinafter "T1014A04" or "T1014G03"), was as follows: loading dose: 20 mg/kg, intravenously 24 hours post injection of tumor cells with maintenance doses of 10 mg/kg, intraperitoneally. Maintenance doses of T1014A04 were given on days four, seven, ten, thirteen and sixteen. Maintenance doses of T1014G03 were given on days four, seven, ten, fourteen, twenty-two and twenty-five. Topotecan was the chemotherapeutic agent used in this experiment. In the experiment with T1014A04, the dose and dosing frequency of Topotecan was as follows: either 0.3 or 0.6 mg/kg, intraperitoneally on the first, second, third, fourth, seventh, tenth, fourteenth, eighteenth, twenty-second, and twenty fifth days of the experiment. In the experiment with T1014G03, the dose and dosing frequency of Topotecan was as follows: either 0.3 or 0.6 mg/kg, intraperitoneally on the first, second, third, fourth, seventh, tenth, thirteenth, and sixteenth days of the experiment.

[0485] When T1014A04 or T1014G03 was administered with topotecan a significant reduction in tumor size was observed. Treatment with the antibody alone may reduce tumor growth at the later time points. (See Figures 1 - 3).

[0486] The above described assay may also be used to test the effect of treatment with more than one anti-TR4 antibody on the growth of tumor cells *in vivo*. For example, animals into which tumor cells have been injected may be treated with both an antibody that specifically binds TR4 and an antibody that specifically binds TR7. As above, this

experiment may be performed in the presence or absence of one or more chemotherapeutic agents. In another variation of the present experiment antibodies of the invention may administered in combination with TRAIL. The ability of such combination therapy to inhibit the growth of tumor cells as compared to treatment with either an antibody alone can be assayed using the methods detailed above, and comparing the results obtained between the combination therapy with the results obtained from treatment with either and anti-TR4 or an anti-TR7 antibody alone.

**Example 7: Effect of Anti-TR4 Antibodies on Human Hepatocytes.**

[0487] The effect of T1014G03 (lot AB22125-M2) in human primary hepatocytes was determined by measuring either caspase activation or cell viability. Human hepatocytes were treated with 15.6, 62.5, 250 or 1000 ng/mL of TRAIL (amino acid residues 114-281, Biomol Research Laboratories Inc, Plymouth Meeting, PA), 62.5, 125, 250, or 1000 ng/ml of isotype control mAb (hIgG<sub>1</sub>, CAT002) or 62.5, 125, 250, or 1000 ng/ml T1014G03. Caspase activation was determined at 6 hrs following treatment, while viability was determined at 24 hrs following treatment.

[0488] Caspase activity was measured using a fluorimetric assay utilizing the caspase substrate Rhodamine conjugated DEVD, (e.g., Homogeneous Fluorimetric Caspases Assay available from Roche Molecular Biochemicals (Indianapolis, IN)). Cell viability was determined using an ALAMAR Blue™ (Biosource International, Camarillo, CA) assay. TRAIL reduced cell viability at all concentrations tested, and induced caspase activity at the highest concentration tested. In contrast to TRAIL, T1014G03 treatment was found not to effect either caspase or cell viability in Human hepatocytes.

**Example 8: RL95-2 Uterine Carcinoma Xenograft Model**

[0489] The objective of this experiment was to examine whether T1014G03 is able to alter the growth pattern of the RL95-2 tumor in athymic mice when T1014G03 is used as a single agent.

[0490] RL95-2 is a uterine adenocarcinoma cell line that forms solid tumors when injected subcutaneously in athymic mice. RL95-2 cells have been demonstrated to express TRAIL-R1 and are sensitive *in vitro* to T1014G03-induced apoptosis in the absence of any sensitizing agent. Based on these findings, the RL95-2 model in athymic mice was selected to test the *in vivo* efficacy of T1014G03 on reduction of pre-existing



tumors. In these experiments, T1014F08 served as a negative control (huIgG $\lambda$ ). T1014F08 binds TRAIL-R1, but was not observed to have agonist activity.

[0491] RL95-2 cells in log phase were injected SC (10 million cells /mouse) in nude mice. After 3 days, the tumor size was determined and the animals were segregated into various treatment groups (6 animals /treatment group) such that all the treatment groups had 5x5 mm size tumor. Mice were injected (IP) with T1014G03 or T1014F08 antibodies at 0.2, 2.0, and 20 mg/Kg doses on days 4, 8, 12, and 16. Tumor size was monitored twice a week from day 3 to day 43. Mice receiving injection vehicle (saline) served as the control. Data were analyzed by non-parametric Mann-Whitney test and are expressed as fold increase in tumor size relative to the day 3-tumor size. The growth of tumor was significantly retarded in mice T1014G03 antibody treatment at 20 mg/Kg compared to the control and T1014F08 treated animals. The effect of T1014G03 antibody at 0.2 and 2.0 mg/Kg was not significantly different from the control. The data demonstrate the ability of T1014G03 to inhibit growth of a pre-established tumor cells.

[0492] The above described assay may also be used to test the effect of treatment with more than one anti-TR4 antibody on the growth of pre-established tumors *in vivo*. For example, animals into which tumor cells have been injected may be treated with both an antibody that specifically binds TR4 and an antibody that specifically binds TR7. As above, this experiment may be performed in the presence of absence of one or more chemotherapeutic agents. In another variation of the present experiment antibodies of the invention may administered in combination with TRAIL. The ability of such combination therapy to inhibit the growth of tumors or even eliminate tumors as compared to treatment with either an antibody alone can be assayed using the methods detailed above, and comparing the results obtained between the combination therapy with the results obtained from treatment with either and anti-TR4 or an anti-TR7 antibody alone.

**Example 9: Immunohistochemistry of primary tumor tissue for expression of TRAIL R1 (TR4) expression.**

[0493] Primary human tumor tissues of the bladder, breast, colon, liver lung ovary and pancreas were stained with a goat anti-human TRAIL-R1 polyclonal antibody (R&D Systems). This antibody stains cells transfected with TRAIL-R1 expression constructs, but not vector control transfected cells. Staining data are presented below in Table 7 below. Positive staining was observed in certain breast, colon, lung, and stomach

carcinoma tissues. In contrast, normal human tissue samples from the same organs, had no specific staining. In addition, no specific staining was observed in normal human and monkey liver and spleen samples.

**Table 7: Immunohistochemical staining of Human tumor and Normal Tissues**

<b>Tumor Tissue</b>	<b># Evaluated</b>	<b>Positive</b>	<b>+/-</b>	<b>Negative</b>
Bladder	2	0	1	1
Breast	2	1	0	1
Colon	2	1	1	0
Liver	2	0	1	1
Lung	2	2	0	1
Ovary	1	0	0	1
Pancreas	2	0	0	2
Stomach	1	1	0	0
<b>Totals</b>	<b>14</b>	<b>5</b>	<b>3</b>	<b>6</b>
<b>Normal Tissue</b>				
Bladder	1	0	0	1
Breast	0	0	0	0
Colon	1	0	0	1
Liver	1	0	1	0
Lung	1	0	0	1
Ovary	1	0	0	1
Pancreas	1	0	0	1
Stomach	0	0	0	0
<b>Totals</b>	<b>6</b>	<b>0</b>	<b>1</b>	<b>5</b>

**Example 10: Antibody Production and Purification**

[0494] The following example describes a large scale antibody production and purification methods that may be used to make antibodies of the present invention. One of skill in the art will be aware of routine modifications to the protocol described below, for example, as regards column choice, column, loading, wash, and elution buffers, and pH.

**Cell Culture Scale-up and Antibody Production**

[0495] A serum-free and animal source-free growth medium (HGS-NS0SF) is used from thawing cells through scale-up to the production bioreactor. The HGS-NS0SF growth medium is prepared by adding 20 mL/L GS supplement and 1 mL/L cholesterol

(synthetic) lipid concentrate into 1 L CD hybridoma media without l-glutamine (Invitrogen/Life technologies). The media are stored at 2-8°C until use.

#### *Thawing cells from MCB Vial(s)*

[0496] Approximately  $16 \times 10^6$  cells are thawed at 37°C in a water bath. The cells are transferred into T-225 culture flask(s) to yield approximately 50 mL working volume with an inoculation density of approximately  $3.0 \times 10^5$  cells/mL. The culture flask(s) is then placed in a humidified CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub> for 4 days.

#### *First Expansion(s) of Culture in Spinner Flask*

[0497] The culture is aseptically expanded into a 500 mL spinner flask to give approximately 300 mL working volume, at an inoculation cell density of approximately  $2.2 \times 10^5$  cells/mL. The spinner flask is then placed on magnetic stirrers in a humidified CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub> for 4 days. The agitation rate for the spinner flask is 80 rpm.

[0498] The culture is again expanded aseptically into one 3000 mL spinner flask to give approximately 1500 mL working volume, at an inoculation cell density of approximately  $2.2 \times 10^5$  cells/mL. The spinner flask is then placed on magnetic stirrers in a humidified CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub> for 4 days. The agitation rate for the spinner flasks is 80 rpm. If a sufficient amount of cell culture is accumulated to inoculate the seed bioreactor, proceed to Step 4. If not, the culture is expanded aseptically into multiple 3000 mL spinner flasks for a total of 3 to 4 expansions, until a sufficient amount of cell culture is accumulated to inoculate the seed bioreactor.

#### *Seed Culture*

[0499] The seed bioreactor is equipped with 2 impellers for mixing, a dissolved oxygen probe, a temperature probe, a pH probe, aseptic sampling and additional systems. The first step of the cell cultivation process is the addition of HGS-NS0SF media into the bioreactor. After the HGS-NS0SF media temperature reaches  $37 \pm 0.5^\circ\text{C}$ , the dissolved oxygen (DO) and pH levels are stabilized by addition of N<sub>2</sub> and CO<sub>2</sub> to decrease dissolved oxygen concentration to  $30 \pm 5\%$  air saturation, and obtain a pH of  $7.20 \pm 0.10$ . The agitation rate is 80 rpm. The pooled cell culture is transferred aseptically to a 15 L seed

bioreactor containing sterile HGS-NS0SF growth media to yield a culture with an inoculation cell density of approximately  $2.2 \times 10^5$  cells/mL. During the cultivation process the temperature is maintained via a heat blanket and a cooling finger, the oxygen concentration is maintained via sparger and surface aeration, and pH is controlled by addition of CO<sub>2</sub> gas to lower the pH. The cultivation period is 5-6 days. The bioreactor air vents are protected by hydrophobic 0.2 µm vent filters.

### ***Production Culture***

[0500] The production bioreactor is equipped with 2 impellers for mixing, 2 dissolved oxygen probes, a temperature probe, 2 pH probes, aseptic sampling and additional systems. 80 L of HGS-NS0SF growth media is aseptically transferred into the 100 L production bioreactor. After the HGS-NS0SF growth media temperature reaches  $37 \pm 0.5^\circ\text{C}$ , the DO and pH levels are stabilized by addition of N<sub>2</sub> and CO<sub>2</sub> to decrease dissolved oxygen concentration to  $30 \pm 5\%$  air saturation, and obtain a pH of  $7.20 \pm 0.10$ . The agitation rate is 45 rpm. The 15 L seed culture is aseptically transferred into the production bioreactor to yield a culture with an inoculation cell density of approximately  $2.2 \times 10^5$  cells/mL. During the cultivation process the temperature is maintained via a heat exchanger, the oxygen concentration is maintained via sparger and surface aeration, and pH is controlled by addition of CO<sub>2</sub> gas to lower the pH. On day 3 after inoculation when cell density reaches approximately  $1.0 \times 10^6$  cells/mL, approximately 6 L of HGS-NS0SF fed-batch media was fed into the production bioreactor. The production culture containing the antibody was harvested on Day 5 after feeding.

### **Recovery and Purification**

#### ***Harvest of Cell Supernatant***

[0501] Cell supernatant, (e.g., culture supernatant from NSO cells expressing antibodies of the invention) is harvested on day 5 or 6 post final feeding in the final production bioreactor using a fed-batch cell culture process. The harvest process is started when the antibody concentration of at least 400 mg/L is attained. Cell culture temperature in the production bioreactor is cooled down to  $15^\circ\text{C}$  at the time of harvest and maintained at that temperature during the recovery. A depth filtration process is used for cell removal and antibody recovery. The filtration process train consists of 4.5 µm, 0.45 µm and 0.2 µm

pore size filters connected in series. A constant flow rate of 1.00 L/min is maintained during the operation with a cross-filter-pressure control of up to 15 psi. The 0.2 µm filtered culture supernatant is collected in a process bag and transferred for purification.

[0502] The purification process is conducted at 22 to 26°C.

#### ***Chromatography on MEP HyperCEL HCIC Column***

[0503] The culture supernatant is loaded onto a MEP HyperCEL™ column, a Hydrophobic charge interaction chromatography, HCIC, available from Ciphergen Biosystems, or equivalent column that is equilibrated in 50 mM Tris, 0.5 M sodium chloride, pH 7.5. The MEP column is washed with 25 mM sodium citrate, 0.15 M sodium chloride, pH 6.4 and eluted with 25 mM sodium citrate, 0.15 M sodium chloride, pH 4.4. The elution is monitored by ultraviolet (UV) absorbance at 280 nm. The peak fractions are collected, analyzed by A<sub>280</sub> and SDS-PAGE. Appropriate fractions are pooled.

#### ***Virus Inactivation***

[0504] The eluate from the MEP column is adjusted with 1 M citric acid to pH 3.4 ± 0.2 and allowed to stand for 45-60 minutes for viral inactivation. The solution is then re-adjusted to pH 5.0 with 1 M Tris base.

#### ***Chromatography on SP Sepharose FF Column***

[0505] The inactivated eluate from the MEP column is diluted with water for injection (WFI) to a conductivity of 5 mS/cm, and loaded onto a SP Sepharose FF (cation exchange chromatography, Amersham-Pharmacia) column, or equivalent column equilibrated with 65 mM sodium acetate, pH 5.0. The antibody is eluted from the SP column with 20 mM sodium citrate, 0.15 M sodium chloride, 1.9% glycine, pH 7.1. The elution is monitored by ultraviolet (UV) absorbance at 280 nm. Peak fractions are collected and analyzed by A<sub>280</sub> and SDS-PAGE. Appropriate fractions are pooled.

#### ***Virus Removal Filtration, Diafiltration and Concentration***

[0506] The eluate from the SP Sepharose FF column is filtered through a sequentially connected 0.2 µm filter and a Pall DV50 viral removal filter. The DV50 filtrate is placed into a 30kD MW cut-off membrane device (Millipore Pellicon) to concentrate to a target concentration of 35-40 mg/mL, and diafiltered against 10 mM

sodium citrate, 1.9% glycine, 0.5% sucrose, pH 6.5. The diafiltered material is monitored by A<sub>280</sub>. The diafiltered bulk is 0.2 µm filtered and stored at 2-8°C up to 24 hours.

#### ***Chromatography on Q Sepharose FF Column***

[0507] The diafiltered TRM-1 solution is passed over a Q Sepharose FF column (anion exchange chromatography, Amersham-Pharmacia) or equivalent column equilibrated with 10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, pH 6.5. The antibody is collected in the flow-through and monitored by A<sub>280</sub>. Appropriate fractions are pooled and the final target concentration is 25 mg/mL.

#### ***Bulk Formulation, Filtration and Bulk Drug Substance Fill***

[0508] Polysorbate 80 (2% stock solution) is pre-filtered through a 0.2 µm filter and added to the antibody solution from step 7 to a final concentration of 0.02%. The purified antibody is aseptically filtered under a laminar flow hood through a 0.2 µm filter and filled into polypropylene containers.

#### ***Storage of Bulk Drug substance***

[0509] The bulk drug substance is stored at 2-8 °C (short-term storage) or at or below -65 °C (long-term storage) prior to the release of the product. In-process testing of the unprocessed production bioreactor culture at harvest for each batch and in-process testing during the purification process are performed. The bioreactor is sampled aseptically and the culture is tested at various times throughout cultivation for cell density, viability and nutrient determination to ensure consistency of material being supplied for purification. The purification process is monitored at each step. Appearance is checked by visual inspection. The protein concentration is determined by Absorbance at 280nm. The pH of the material is checked. Purity is checked, for example, by SDS-PAGE and size exclusion chromatography. An ELISA may be performed to check the ability of the antibody to bind its antigen. The biological activity of the antibody is also monitored. Residual DNA content, Endotoxin levels, and the bioburden (the number of viable organisms present in the antibody preparation) are all monitored and kept at or below standard acceptable levels. Additionally the oligosaccharide content may be analyzed; the peptide sequence of the antibody chains may also be analyzed using N-terminal sequencing and peptide mapping. Short and long-term studies of antibody stability may

also be performed.

**[0511]** It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

**[0512]** The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

**[0513]** Further, the Sequence Listing submitted herewith, in both computer and paper forms, is hereby incorporated by reference in its entirety.

**[0514]** The entire disclosure (including the specification, sequence listing, and drawings) of each of the following U.S. applications are herein incorporated by reference in their entirety: U.S. Provisional Application Serial Numbers 60/369,860 filed April 5, 2002; 60/341,237 filed December 20, 2001; 60/331,310 filed November 14, 2001; 60/331,044 filed November 7, 2001; 60/327,364 filed October 9, 2001; 60/323,807 filed September 21, 2001; 60/309,176 filed August 2, 2001; 60/294,981 filed June 4, 2001; and 60/293,473 filed May 25, 2001.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 18, Table 1.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

July 30, 2001

Accession Number

PTA-3571

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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**ATCC Deposit No. PTA-3571****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**ATCC Deposit No.: PTA-3571****UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**DENMARK**

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**SWEDEN**

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**NETHERLANDS**

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 18, Table 1.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

July 30, 2001

Accession Number

PTA-3570

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

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**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

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**ATCC Deposit No. PTA-3570****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**ATCC Deposit No.: PTA-3570**

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

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## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 18, Table 1.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*  
10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

August 29, 2001

Accession Number

PTA-3675

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

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**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

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**ATCC Deposit No. PTA-3675****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**FINLAND**

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**ATCC Deposit No.: PTA-3675**

## **UNITED KINGDOM**

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## **DENMARK**

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## **NETHERLANDS**

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 19, paragraph 62.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*  
10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

January 21, 1997

Accession Number

97853

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

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**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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**ATCC Deposit No. 97853****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**ATCC Deposit No.: 97853**

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

## **DENMARK**

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## **SWEDEN**

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## **NETHERLANDS**

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

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**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

November 20, 1996

Accession Number

97798

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

Europe

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**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

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<b>Jeryl McDowell</b> <b>PCT/Int'l Appl Processing Div.</b> <b>(703) 305-3639</b>			Authorized officer		

**ATCC Deposit No. 97798****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

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**ATCC Deposit No.: 97798**

## **UNITED KINGDOM**

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## **DENMARK**

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## **SWEDEN**

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## **NETHERLANDS**

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

**A.** The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 19, paragraph 62.

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

March 7, 1997

Accession Number

97920

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)*

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

**E. SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

	For receiving Office use only			For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received with the international application <b>Jeryl McBowell</b> <b>PCT/Internet App. Processing Div.</b> <b>(703) 305-3039</b>			<input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer		

**ATCC Deposit No. 97920****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.



**ATCC Deposit No.: 97920**

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

## **DENMARK**

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Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard  
Manassas, Virginia 20110-2209  
United States of America

Date of deposit

May 15, 1997

Accession Number

209040

**C. ADDITIONAL INDICATIONS** (*leave blank if not applicable*)

This information is continued on an additional sheet ☐

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

**E. SEPARATE FURNISHING OF INDICATIONS** (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

	For receiving Office use only			For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received with the international application			<input type="checkbox"/> This sheet was received by the International Bureau on:		
Authorized officer <i>Jerry McDowell</i> Patent & Appl Processing Div. (202) 305-3000			Authorized officer		

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The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

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**ATCC Deposit No.: 209040**

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WHAT IS CLAIMED IS:

1. An isolated antibody or fragment thereof comprising a first amino acid sequence at least 95% identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of either VHCDR1, VHCDR2, or VHCDR3 of any one of SEQ ID NOS:42-53; and

(b) the amino acid sequence of either VLCDR1, VLCDR2, or VLCDR3 of any one of SEQ ID NOS:42-53;

wherein said antibody or fragment thereof immunospecifically binds TR4.

2. The antibody or fragment thereof of claim 1, wherein the second amino acid sequence consists of the amino acid sequence of a VHCDR3 of any one of SEQ ID NOS: 42-53.

3. The antibody or fragment thereof of claim 1, that preferentially binds TR4 relative to its ability to bind TR1, TR5, TR7 and TR10.

4. The antibody or fragment thereof of claim 1, that binds TR4 expressed on the surface of a cell.

5. An isolated antibody or fragment thereof comprising:

(a) an amino acid sequence that is at least 90% identical to a VH domain of any one of SEQ ID NOS: 42-53;

(b) an amino acid sequence that is at least 90% identical to a VL domain of any one of SEQ ID NOS: 42-53; or

(c) both (a) and (b);

wherein said antibody or fragment thereof immunospecifically binds TR4.

6. The antibody or fragment thereof of claim 5, wherein the VH domain has the amino acid sequence of the VH domain of SEQ ID NO:42 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:42.

7. The antibody or fragment thereof of claim 5, wherein the VH domain has the amino acid sequence of the VH domain of SEQ ID NO:43 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:43.

8. The antibody or fragment thereof of claim 5, wherein the VH domain has the amino acid sequence of the VH domain of SEQ ID NO:48 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:48.

9. The antibody or fragment thereof of claim 5, that preferentially binds TR4 relative to its ability to bind TR1, TR5, TR7 and TR10.

10. The antibody or fragment thereof of claim 5, that binds TR4 expressed on the surface of a cell.

11. The antibody or fragment thereof of claim 5 comprising:  
(a) the amino acid sequence of a VH domain of any one of SEQ ID NOS:42-53;  
(b) the amino acid sequence of a VL domain of any one of SEQ ID NOS:42-53; or  
(c) both (a) and (b);

wherein said antibody or fragment thereof immunospecifically binds TR4.

12. The antibody or fragment thereof of claim 11, wherein the VH domain has the amino acid sequence of the VH domain SEQ ID NO:42 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:42.

13. The antibody or fragment thereof of claim 11, wherein the VH domain has the amino acid sequence of the VH domain SEQ ID NO:43 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:43.

14. The antibody or fragment thereof of claim 11, wherein the VH domain has the amino acid sequence of the VH domain SEQ ID NO:48 and the VL domain has the amino acid sequence of the VL domain of SEQ ID NO:48.

15. The antibody or fragment thereof of claim 11, that preferentially binds TR4 relative to its ability to bind TR1, TR5, TR7 and TR10.

16. The antibody or fragment thereof of claim 11, that binds TR4 expressed on the surface of a cell.

17. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is selected from the group consisting of:

- (a) a whole immunoglobulin molecule;
- (b) an scFv;
- (c) a monoclonal antibody;
- (d) a human antibody;
- (e) a chimeric antibody;
- (f) a humanized antibody;
- (g) a Fab fragment;
- (h) an Fab' fragment;
- (i) an F(ab')<sub>2</sub>;
- (j) an Fv; and
- (k) a disulfide linked Fv.

18. The antibody or fragment thereof of claim 5 which comprises a heavy chain immunoglobulin constant domain selected from the group consisting of:

- (a) a human IgM constant domain;
- (b) a human IgG1 constant domain;
- (c) a human IgG2 constant domain;
- (d) a human IgG3 constant domain;
- (e) a human IgG4 constant domain; and
- (f) a human IgA constant domain.

19. The antibody or fragment thereof of claim 5 which comprises a light chain immunoglobulin constant domain selected from the group consisting of:

- (a) a human Ig kappa constant domain; and
- (b) a human Ig lambda constant domain.

20. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof has a dissociation constant ( $K_D$ ) selected from the group consisting of:

- (a) a dissociation constant ( $K_D$ ) between  $10^{-7}$  M (inclusive) and  $10^{-8}$  M;
- and
- (b) a dissociation constant ( $K_D$ ) between  $10^{-8}$  M (inclusive) and  $10^{-9}$  M.

21. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof has a dissociation constant ( $K_D$ ) of less than or equal to  $10^{-9}$  M.

22. The antibody or fragment thereof of claim 21 wherein the antibody or fragment thereof has a  $K_D$  between  $10^{-9}$  M and  $10^{-10}$  M.

23. The antibody or fragment thereof of claim 21 wherein the antibody or fragment thereof has a  $K_D$  between  $10^{-10}$  M (inclusive) and  $10^{-11}$  M.

24. The antibody or fragment thereof of claim 21 wherein the antibody or fragment thereof has a  $K_D$  between  $10^{-11}$  M (inclusive) and  $10^{-12}$  M.

25. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is conjugated to a detectable label.

26. The antibody or fragment thereof of claim 25, wherein the detectable label is a radiolabel.

27. The antibody or fragment thereof of claim 26, wherein the radiolabel is  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ ,  $^{177}\text{Lu}$ ,  $^{166}\text{Ho}$ , or  $^{153}\text{Sm}$ .

28. The antibody or fragment thereof of claim 25, wherein the detectable label is an enzyme, a fluorescent label, a luminescent label, or a bioluminescent label.

29. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is biotinylated.



30. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is conjugated to a therapeutic or cytotoxic agent.

31. The antibody or fragment thereof of claim 30, wherein the therapeutic or cytotoxic agent is selected from the group consisting of:

- (a) an anti-metabolite;
- (b) an alkylating agent;
- (c) an antibiotic;
- (d) a growth factor;
- (e) a cytokine;
- (f) an anti-angiogenic agent;
- (g) an anti-mitotic agent;
- (h) an anthracycline;
- (i) toxin; and
- (j) an apoptotic agent.

32. The antibody or fragment thereof of any one of claim 5 wherein the antibody or fragment thereof is attached to a solid support.

33. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof immunospecifically binds TR4 in a Western blot.

34. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof immunospecifically binds TR4 in an ELISA.

35. An isolated cell that produces the antibody or fragment thereof of claim 11.

36. The antibody or fragment thereof of claim 5 that does not inhibit the ability of TRAIL to bind TR4.

37. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is an agonist of TR4.

38. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof stimulates apoptosis of TR4 expressing cells.

39. The antibody or fragment thereof of claim 38 wherein the antibody or fragment thereof stimulates apoptosis of TR4 expressing cells better than an equal concentration of TRAIL polypeptide stimulates apoptosis of TR4 expressing cells.

40. The antibody or fragment thereof of claim 38 wherein the antibody or fragment thereof stimulates apoptosis of TR4 expressing cells equally well in the presence or absence of antibody cross-linking reagents.

41. The antibody or fragment thereof of claim 38 wherein the antibody or fragment thereof is not hepatotoxic.

42. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof upregulates TRAIL receptor expression.

43. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof inhibits TRAIL binding to TR4.

44. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof is an antagonist of TR4.

45. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof inhibits apoptosis of TR4 expressing cells.

46. The antibody or fragment thereof of claim 5 wherein the antibody or fragment thereof downregulates TRAIL receptor expression.

47. An antibody or fragment thereof that binds the same epitope on a TR4 polypeptide as an antibody or fragment thereof of claim 11.

48. An antibody or fragment thereof that binds the same epitope on a TR4 polypeptide as an antibody or fragment thereof of claim 12.

49. An antibody or fragment thereof that binds the same epitope on a TR4 polypeptide as an antibody or fragment thereof of claim 13.

50. An antibody or fragment thereof that binds the same epitope on a TR4 polypeptide as an antibody or fragment thereof of claim 14.

51. The antibody or fragment thereof of claim 5 in a pharmaceutically acceptable carrier.

52. A method of treating, preventing or ameliorating a cancer comprising administering to an animal the antibody or fragment thereof of claim 5 or a composition containing said antibody or fragment thereof.

53. The method of claim 52 wherein the animal is a human.

54. The method of claim 52 wherein the cancer is colon cancer.

55. The method of claim 52 wherein the cancer is breast cancer.

56. The method of claim 52 wherein the cancer is uterine cancer.

57. The method of claim 52 wherein the cancer is pancreatic cancer.

58. The method of claim 52 wherein the cancer is lung cancer.

59. The method of claim 52 wherein the cancer is gastrointestinal cancer.

60. The method of claim 52 wherein the cancer is Kaposi's sarcoma.

61. The method of claim 52 wherein the cancer is a cancer of the central nervous system.

62. The method of claim 61 wherein the cancer of the central nervous system is a medulloblastoma.

63. The method of claim 61 wherein the cancer of the central nervous system is a neuroblastoma.

64. The method of claim 61 wherein the cancer of the central nervous system is a glioblastoma.

65. The method of claim 52, wherein the antibody or fragment thereof is administered in combination with a chemotherapeutic agent.

66. The method of claim 65, wherein chemotherapeutic agent is selected from the group consisting of:

- (a) irinotecan;
- (b) paclitaxel (TAXOL)®; and
- (c) gemcitabine.

67. A method of treating, preventing or ameliorating a disease or disorder selected from the group consisting of:

- (a) graft vs. host disease (GVHD);
- (b) AIDS; and
- (c) a neurodegenerative disorder;

comprising administering to an animal the antibody or fragment thereof of claim 5 or a composition containing said antibody or portion thereof.

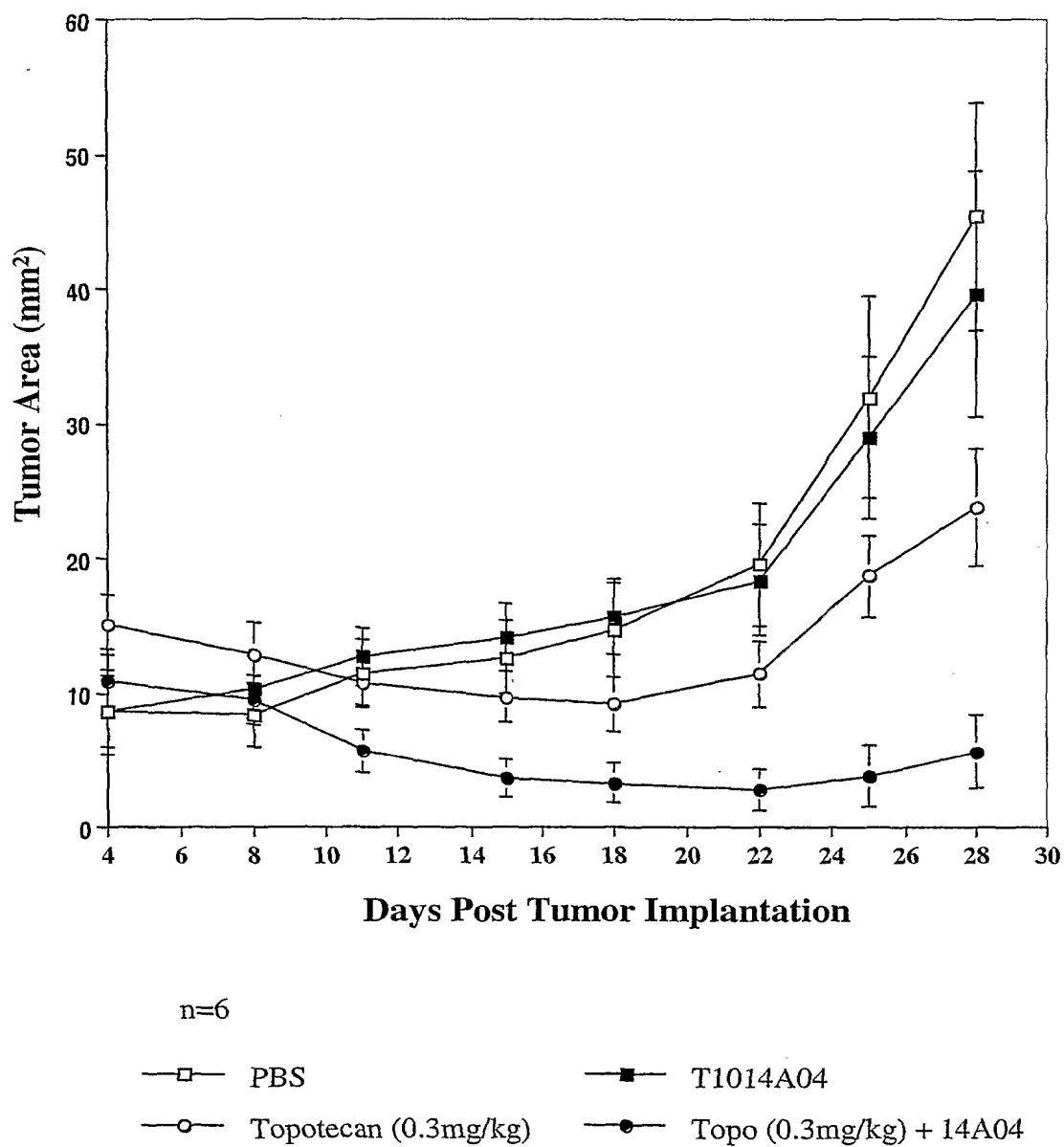
68. The method of claim 67, wherein the animal is a human.

69. A method of inhibiting the growth of or killing TR4 expressing cells, comprising administering to an animal in which such inhibition of growth or killing of TR4 receptor expressing cells is desired, the antibody or fragment thereof of claim 5 or a composition containing said antibody or fragment thereof in an amount effective to inhibit the growth of or kill TR4 expressing cells.

70. A method of detecting expression of a TR4 polypeptide comprising:
- (a) assaying the expression of a TR4 polypeptide in a biological sample from an individual using the antibody or fragment thereof of claim 5; and
  - (b) comparing the level of a TR4 polypeptide with a standard level of a TRAIL receptor polypeptide.
71. A method of detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders comprising:
- (a) assaying the expression of a TR4 polypeptide in a biological sample from an individual using the antibody or fragment thereof of claim 5; and
  - (b) comparing the level of a TR4 polypeptide with a standard level of TR4 polypeptide.
72. A kit comprising the antibody or fragment thereof of claim 5.
73. The kit of claim 72 comprising a control antibody.
74. The kit of claim 72, wherein the antibody or fragment thereof is coupled or conjugated to a detectable label.
75. The antibody expressed by the cell line of ATCC Deposit PTA-3570.
76. The antibody expressed by the cell line of ATCC Deposit PTA-3571.
77. The antibody expressed by the cell line of ATCC Deposit PTA-3675.

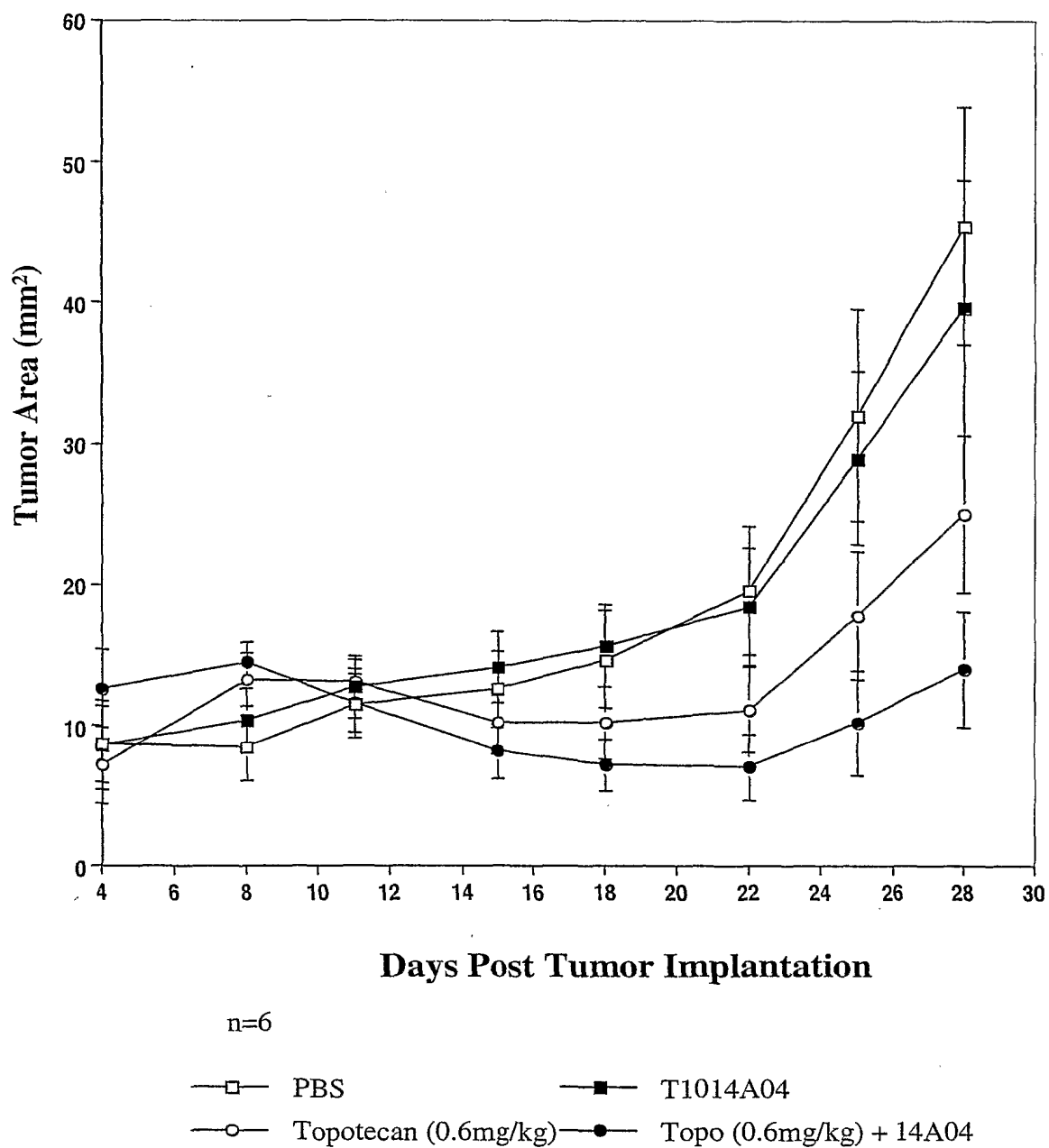
1/3

**Effect of T1014A04 on SW480 tumor  
growth in Swiss Nu/Nu mice**

**FIG. 1**

2/3

**Effect of T1014A04 on SW480 tumor growth  
in Swiss Nu/Nu mice**

**FIG. 2**

3/3

Effect of 14G03 treatment on the growth of SW480 tumors  
*in vivo* after 28 days

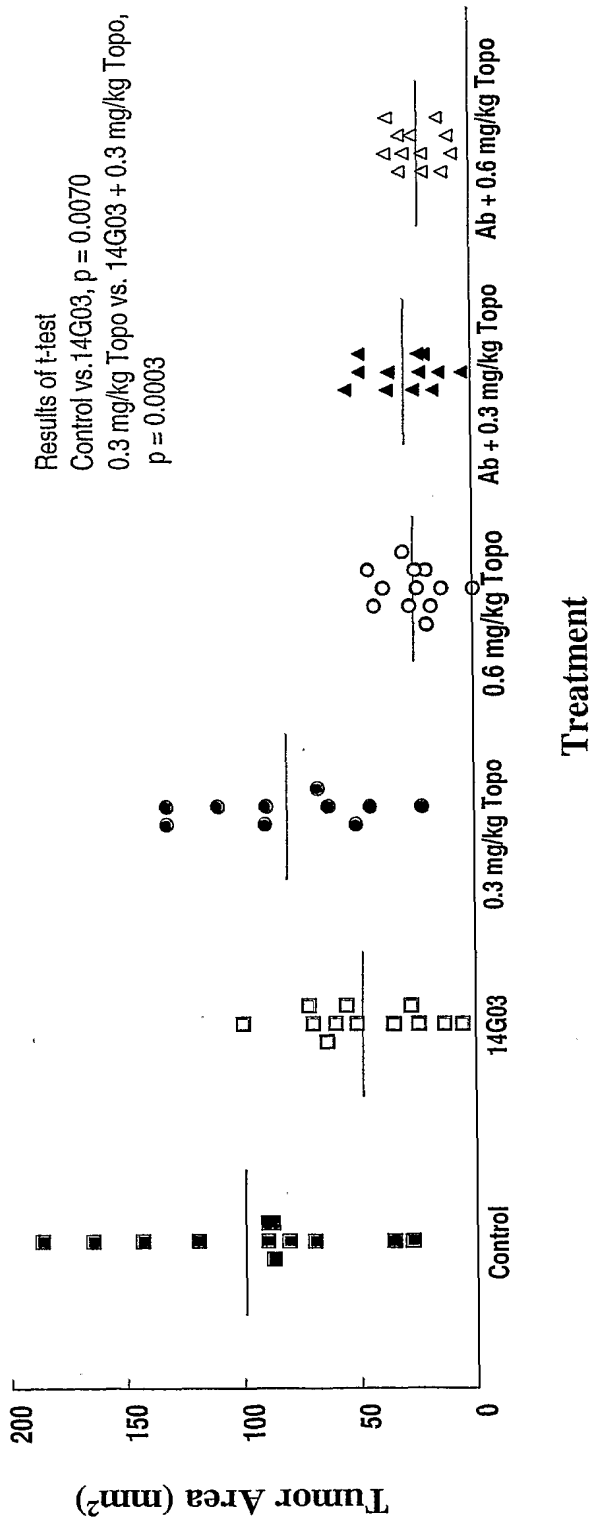


FIG. 3



## SEQUENCE LISTING

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Receptors

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<151> 2002-04-05

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<151> 2001-05-25

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<170> PatentIn Ver. 2.1

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 Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met Leu Met Lys Trp Val  
 405 410 415  
 Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr Leu Leu Asp Ala Leu  
 420 425 430  
 Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys Ile Gln Asp Leu Leu  
 435 440 445  
 Val Asp Ser Gly Lys Phe Ile Tyr Leu Glu Asp Gly Thr Gly Ser Ala  
 450 455 460  
 Val Ser Leu Glu  
 465

<210> 2  
 <211> 299  
 <212> PRT  
 <213> Homo sapiens

<400> 2  
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 1 5 10 15  
 Arg Ala Pro Arg Pro Pro Asp Gly Arg Gly Arg Val Arg Pro Arg Thr  
 20 25 30  
 Gln Asp Gly Val Gly Asn His Thr Met Ala Arg Ile Pro Lys Thr Leu  
 35 40 45  
 Lys Phe Val Val Val Ile Val Ala Val Leu Leu Pro Val Leu Ala Tyr  
 50 55 60  
 Ser Ala Thr Thr Ala Arg Gln Glu Glu Val Pro Gln Gln Thr Val Ala  
 65 70 75 80  
 Pro Gln Gln Gln Arg His Ser Phe Lys Gly Glu Glu Cys Pro Ala Gly  
 85 90 95  
 Ser His Arg Ser Glu His Thr Gly Ala Cys Asn Pro Cys Thr Glu Gly  
 100 105 110  
 Val Asp Tyr Thr Asn Ala Ser Asn Asn Glu Pro Ser Cys Phe Pro Cys  
 115 120 125  
 Thr Val Cys Lys Ser Asp Gln Lys His Lys Ser Ser Cys Thr Met Thr  
 130 135 140  
 Arg Asp Thr Val Cys Gln Cys Lys Glu Gly Thr Phe Arg Asn Glu Asn  
 145 150 155 160  
 Ser Pro Glu Met Cys Arg Lys Cys Ser Arg Cys Pro Ser Gly Glu Val  
 165 170 175  
 Gln Val Ser Asn Cys Thr Ser Trp Asp Asp Ile Gln Cys Val Glu Glu

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                180                185                190
Phe Gly Ala Asn Ala Thr Val Glu Thr Pro Ala Ala Glu Glu Thr Met
    195                200                205

Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Asn
    210                215                220

Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr
    225                230                235                240

Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser
    245                250                255

Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro
    260                265                270

Gly Thr Pro Ala Ser Ser His Tyr Leu Ser Cys Thr Ile Val Gly Ile
    275                280                285

Ile Val Leu Ile Val Leu Leu Ile Val Phe Val
    290                295

<210> 3
<211> 411
<212> PRT
<213> Homo sapiens

<400> 3
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
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Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
    20                25                30

Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
    35                40                45

Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
    50                55                60

Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
    65                70                75                80

Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
    85                90                95

Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu Leu Phe
    100                105                110

Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
    115                120                125

Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                135                140

Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
    145                150                155                160

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Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile  
 165 170 175  
 Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala  
 180 185 190  
 Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp  
 195 200 205  
 Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly  
 210 215 220  
 Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp  
 225 230 235 240  
 Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro  
 245 250 255  
 Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn  
 260 265 270  
 Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala  
 275 280 285  
 Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp  
 290 295 300  
 Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val  
 305 310 315 320  
 Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp  
 325 330 335  
 Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr  
 340 345 350  
 Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala  
 355 360 365  
 Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu  
 370 375 380  
 Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met  
 385 390 395 400  
 Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser  
 405 410

&lt;210&gt; 4

&lt;211&gt; 386

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 4

Met Gly Leu Trp Gly Gln Ser Val Pro Thr Ala Ser Ser Ala Arg Ala  
 1 5 10 15

Gly Arg Tyr Pro Gly Ala Arg Thr Ala Ser Gly Thr Arg Pro Trp Leu  
 20 25 30

Leu Asp Pro Lys Ile Leu Lys Phe Val Val Phe Ile Val Ala Val Leu  
 35 40 45  
 Leu Pro Val Arg Val Asp Ser Ala Thr Ile Pro Arg Gln Asp Glu Val  
 50 55 60  
 Pro Gln Gln Thr Val Ala Pro Gln Gln Gln Arg Arg Ser Leu Lys Glu  
 65 70 75 80  
 Glu Glu Cys Pro Ala Gly Ser His Arg Ser Glu Tyr Thr Gly Ala Cys  
 85 90 95  
 Asn Pro Cys Thr Glu Gly Val Asp Tyr Thr Ile Ala Ser Asn Asn Leu  
 100 105 110  
 Pro Ser Cys Leu Leu Cys Thr Val Cys Lys Ser Gly Gln Thr Asn Lys  
 115 120 125  
 Ser Ser Cys Thr Thr Thr Arg Asp Thr Val Cys Gln Cys Glu Lys Gly  
 130 135 140  
 Ser Phe Gln Asp Lys Asn Ser Pro Glu Met Cys Arg Thr Cys Arg Thr  
 145 150 155 160  
 Gly Cys Pro Arg Gly Met Val Lys Val Ser Asn Cys Thr Pro Arg Ser  
 165 170 175  
 Asp Ile Lys Cys Lys Asn Glu Ser Ala Ala Ser Ser Thr Gly Lys Thr  
 180 185 190  
 Pro Ala Ala Glu Glu Thr Val Thr Thr Ile Leu Gly Met Leu Ala Ser  
 195 200 205  
 Pro Tyr His Tyr Leu Ile Ile Ile Val Val Leu Val Ile Ile Leu Ala  
 210 215 220  
 Val Val Val Val Gly Phe Ser Cys Arg Lys Lys Phe Ile Ser Tyr Leu  
 225 230 235 240  
 Lys Gly Ile Cys Ser Gly Gly Gly Gly Gly Pro Glu Arg Val His Arg  
 245 250 255  
 Val Leu Phe Arg Arg Arg Ser Cys Pro Ser Arg Val Pro Gly Ala Glu  
 260 265 270  
 Asp Asn Ala Arg Asn Glu Thr Leu Ser Asn Arg Tyr Leu Gln Pro Thr  
 275 280 285  
 Gln Val Ser Glu Gln Glu Ile Gln Gly Gln Glu Leu Ala Glu Leu Thr  
 290 295 300  
 Gly Val Thr Val Glu Ser Pro Glu Glu Pro Gln Arg Leu Leu Glu Gln  
 305 310 315 320  
 Ala Glu Ala Glu Gly Cys Gln Arg Arg Arg Leu Leu Val Pro Val Asn  
 325 330 335  
 Asp Ala Asp Ser Ala Asp Ile Ser Thr Leu Leu Asp Ala Ser Ala Thr  
 340 345 350

Leu Glu Glu Gly His Ala Lys Glu Thr Ile Gln Asp Gln Leu Val Gly  
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Ser Glu Lys Leu Phe Tyr Glu Glu Asp Glu Ala Gly Ser Ala Thr Ser  
 370 375 380

Cys Leu  
 385

<210> 5

<211> 401

<212> PRT

<213> Homo sapiens

<400> 5

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser Ile  
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Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp  
 20 25 30

Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro Pro Gly Thr  
 35 40 45

Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr Val Cys Ala Pro  
 50 55 60

Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His Thr Ser Asp Glu Cys  
 65 70 75 80

Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu Gln Tyr Val Lys Gln Glu  
 85 90 95

Cys Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr  
 100 105 110

Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe  
 115 120 125

Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg  
 130 135 140

Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys  
 145 150 155 160

Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys  
 165 170 175

Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr  
 180 185 190

Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg  
 195 200 205

Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val  
 210 215 220

Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile

225		230		235		240
Lys Arg Gln His	Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu					
	245			250		255
Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln						
	260			265		270
Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala						
	275			280		285
Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly						
	290			295		300
Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys						
305		310		315		320
Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn						
	325			330		335
Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser						
	340			345		350
Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr						
	355			360		365
Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu						
	370			375		380
Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys						
385		390		395		400

Leu

&lt;210&gt; 6

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer useful for amplifying VH and VL domains

&lt;400&gt; 6

caggtgcagc tgggtgcagtc tgg

23

&lt;210&gt; 7

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer useful for amplifying VH and VL domains

&lt;400&gt; 7

caggtcaact taagggagtc tgg

23

&lt;210&gt; 8

&lt;211&gt; 23



<212> DNA  
 <213> Artificial sequence  
  
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 <400> 12  
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 <210> 13  
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<212> DNA  
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<400> 13  
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<210> 14  
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<400> 14  
tgaggagacg gtgaccaggg ttcc 24  
  
<210> 15  
<211> 24  
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<223> PCR primer useful for amplifying VH and VL domains  
  
<400> 15  
tgaggagacg gtgaccgtgg tccc 24  
  
<210> 16  
<211> 23  
<212> DNA  
<213> Artificial sequence  
  
<220>  
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<400> 16  
gacatccaga tgacccagtc tcc 23  
  
<210> 17  
<211> 23  
<212> DNA  
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<223> PCR primer useful for amplifying VH and VL domains  
  
<400> 17  
gatgttgtga tgactcagtc tcc 23  
  
<210> 18  
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<212> DNA  
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<220>  
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<400> 18  
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<210> 19  
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<223> PCR primer useful for amplifying VH and VL domains

<400> 19  
gaaattgtgt tgacgcagtc tcc 23

<210> 20  
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<212> DNA  
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<400> 20  
gacatcgtga tgaccagtc tcc 23

<210> 21  
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<400> 21  
gaaacgacac tcacgcagtc tcc 23

<210> 22  
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<400> 22  
gaaattgtgc tgactcagtc tcc 23

<210> 23  
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<212> DNA  
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<220>  
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<400> 23  
cagtctgtgt tgacgcagcc gcc 23

<210> 24  
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<212> DNA  
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<400> 24  
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<210> 25  
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<220>  
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<400> 25  
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<210> 26  
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<212> DNA  
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<220>  
<223> PCR primer useful for amplifying VH and VL domains

<400> 26  
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<210> 27  
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<212> DNA  
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<220>  
<223> PCR primer useful for amplifying VH and VL domains

<400> 27  
cacgttatac tgactcaacc gcc 23

<210> 28  
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<212> DNA  
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 <400> 28  
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 <210> 29  
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 <400> 29  
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 <210> 30  
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 <400> 30  
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 <210> 31  
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 <400> 31  
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 <210> 32  
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 <400> 32  
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 <210> 33  
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<212> DNA  
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 <400> 33  
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 <210> 34  
 <211> 24  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 34  
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 <210> 35  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 35  
 cagtctgtgt tgacgcagcc gcc 23  
  
 <210> 36  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 36  
 cagtctgccc tgactcagcc tgc 23  
  
 <210> 37  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
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 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 37  
 tcctatgtgc tgactcagcc acc 23  
  
 <210> 38  
 <211> 23

<212> DNA  
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 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 38  
 tcttctgagc tgactcagga ccc 23  
  
 <210> 39  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 39  
 cacggttatac tgactcaacc gcc 23  
  
 <210> 40  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 40  
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 <210> 41  
 <211> 23  
 <212> DNA  
 <213> Artificial sequence  
  
 <220>  
 <223> PCR primer useful for amplifying VH and VL domains  
  
 <400> 41  
 aattttatgc tgactcagcc cca 23  
  
 <210> 42  
 <211> 245  
 <212> PRT  
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 <220>  
 <223> T1014A04 scFv  
  
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 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ile Ser Gly Asp Ser Phe Asn Ala Tyr  
 20 25 30

<400> 43  
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Met Pro Gly Ala  
1 5 10 15  
Ser Val Lys Leu Ser Cys Arg Val Ser Gly Asp Thr Phe Thr Ala Tyr  
20 25 30  
Phe Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45



Gly Trp Phe Asn Pro Ile Ser Gly Thr Ala Gly Ser Ala Glu Lys Phe  
 50 55 60  
 Arg Gly Arg Val Ala Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Asn Arg Leu Thr Phe Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Gln His Arg Gly Asn Thr Phe Asp Pro Trp Gly Gln Gly Thr  
 100 105 110  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 115 120 125  
 Gly Gly Gly Gly Ser Ala Gln Ser Ala Leu Thr Gln Pro Ala Ser Val  
 130 135 140  
 Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser  
 145 150 155 160  
 Ser Asp Ile Gly Ala Tyr Lys Tyr Val Ser Trp Tyr Gln Gln His Pro  
 165 170 175  
 Gly Lys Ala Pro Lys Leu Val Ile Tyr Glu Val Ser Asn Arg Pro Ser  
 180 185 190  
 Gly Val Ser Ser Arg Phe Ser Gly Ser Lys Ser Gly Gln Thr Ala Ser  
 195 200 205  
 Leu Thr Ile Ser Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys  
 210 215 220  
 Asn Ser Tyr Gln Gly Tyr Asn Thr Trp Val Phe Gly Gly Gly Thr Lys  
 225 230 235 240  
 Val Thr Val Leu Gly  
 245

<210> 44  
 <211> 244  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> T1014A02 scFv

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 1 5 10 15  
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Asp Tyr  
 20 25 30  
 Tyr Trp Ser Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45  
 Gly Ser Ile Asp Tyr Ala Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys

50                                      55                                      60  
 Ser Arg Val Thr Met Thr Ile Asp Lys Ser Lys Lys Gln Phe Pro Leu  
 65                                      70                                      75                                      80  
 Lys Ile Asp Ser Val Thr Ala Ala Asp Thr Ala Met Tyr Tyr Cys Ala  
 85                                      90                                      95  
 Arg Gln Leu Gly Arg Ile Ser Asp Tyr Trp Gly Gln Gly Thr Leu Val  
 100                                      105                                      110  
 Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly  
 115                                      120                                      125  
 Gly Gly Ser Ala Leu Ser Tyr Val Leu Thr Gln Pro Pro Ser Ala Ser  
 130                                      135                                      140  
 Gly Thr Pro Gly Gln Arg Val Thr Ile Ser Cys Ala Gly Ser Ser Ser  
 145                                      150                                      155                                      160  
 Asn Ile Gly Gly Asn Thr Val Asn Trp Tyr Gln Gln Leu Pro Ala Thr  
 165                                      170                                      175  
 Ala Pro Lys Leu Leu Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val  
 180                                      185                                      190  
 Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala  
 195                                      200                                      205  
 Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Thr  
 210                                      215                                      220  
 Trp Asp Asp Ser Arg Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Leu  
 225                                      230                                      235                                      240  
 Thr Val Leu Gly  
 <210> 45  
 <211> 245  
 <212> PRT  
 <213> Artificial sequence  
 <220>  
 <223> T1014A12 scFv  
 <400> 45  
 Glu Val Gln Leu Val Gln Ser Gly Ala Asp Val Lys Arg Pro Gly Ala  
 1                                      5                                      10                                      15  
 Ser Val Lys Val Ser Cys Lys Ile Ser Gly Asp Ser Phe Thr Ala Tyr  
 20                                      25                                      30  
 Phe Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35                                      40                                      45  
 Gly Trp Phe Asn Pro Asp Ser Gly Thr Ala Asp Ser Ala Gln Lys Phe  
 50                                      55                                      60  
 His Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ser Ser Thr Ala Phe  
 65                                      70                                      75                                      80

[illegible]

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<210> 46
<211> 245
<212> PRT
<213> Artificial sequence
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<220>  
<223> T1014B01 scFv

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Ser	Val	Lys	Val	Ser	Cys	Lys	Ile	Ser	Gly	Asp	Thr	Phe	Ala	Ala	Tyr
			20					25					30		
Phe	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			
Gly	Trp	Phe	Asn	Pro	Asn	Ser	Gly	Thr	Ala	Asp	Ser	Ser	Gln	Lys	Phe
	50					55					60				
His	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Ile	Ser	Thr	Ala	Tyr
65					70					75					80
Met	Glu	Leu	Ser	Arg	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala Arg Gln His Arg Ser Asn Thr Phe Asp Pro Trp Gly Gln Gly Thr  
100 105 110

Met Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser  
115 120 125

Gly Gly Gly Gly Ser Ala Gln Ser Val Val Thr Gln Pro Pro Ser Val  
130 135 140

Ser Gly Ser Pro Gly Gln Ser Val Thr Ile Ser Cys Thr Gly Thr Ser  
145 150 155 160

Ser Asp Ile Gly Ala Tyr Asn Tyr Val Ser Trp Phe Gln Gln His Pro  
165 170 175

Gly Lys Ala Pro Lys Leu Ile Ile Ser Glu Val Ser Lys Arg Pro Ser  
180 185 190

Gly Val Pro Asp Arg Leu Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser  
195 200 205

Leu Thr Val Ser Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys  
210 215 220

Gly Ser Tyr Ala Gly Ser Asn Ile Trp Val Phe Gly Gly Gly Thr Lys  
225 230 235 240

Val Thr Val Leu Gly  
245

&lt;210&gt; 47

&lt;211&gt; 245

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; T1014B11 scFv

&lt;400&gt; 47

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ile Ser Gly Asp Ser Phe Thr Ala Tyr  
20 25 30

Phe Ile His Trp Leu Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Met  
35 40 45

Gly Trp Phe Asn Pro Ile Ser Gly Thr Ala Gly Ser Pro Gln Lys Phe  
50 55 60

His Gly Arg Val Ala Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Thr Arg Leu Ala Ser Asp Asp Thr Ala Ile Tyr Tyr Cys  
85 90 95

Ala Arg Gln His His Ser Asn Thr Phe Asp Pro Trp Gly Gln Gly Thr  
100 105 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
           115                                  120                                  125  
 Gly Gly Gly Gly Ser Ala Gln Ser Ala Leu Thr Gln Pro Ala Ser Val  
           130                                  135                                  140  
 Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly Thr Asn  
 145                                  150                                  155                                  160  
 Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr Gln Gln His Pro  
                                   165                                  170                                  175  
 Gly Lys Ala Pro Lys Leu Met Ile Tyr Glu Val Asn Asn Arg Pro Ser  
                                   180                                  185                                  190  
 Gly Val Ser Asn Arg Phe Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser  
           195                                  200                                  205  
 Leu Thr Ile Ser Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys  
           210                                  215                                  220  
 Ser Ser Tyr Thr Thr Ser Asn Thr Trp Val Phe Gly Gly Gly Thr Lys  
 225                                  230                                  235                                  240  
 Leu Thr Val Leu Gly  
                                   245

&lt;210&gt; 48

&lt;211&gt; 245

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; T1014F11 scFv

&lt;400&gt; 48

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
   1                                  5                                  10                                  15  
 Ser Val Lys Leu Ser Cys Arg Val Ser Gly Asp Thr Phe Thr Ala Tyr  
           20                                  25                                  30  
 Phe Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Pro Glu Trp Met  
           35                                  40                                  45  
 Gly Trp Phe Asn Pro Ile Ser Gly Thr Ala Gly Ser Ala Ala Arg Phe  
           50                                  55                                  60  
 Arg Gly Arg Val Ala Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr  
           65                                  70                                  75                                  80  
 Met Glu Leu Asn Arg Leu Thr Phe Asp Asp Thr Ala Val Tyr Tyr Cys  
                                   85                                  90                                  95  
 Ala Arg Gln His Arg Gly Asn Thr Phe Asp Pro Trp Gly Lys Gly Thr  
           100                                  105                                  110  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
           115                                  120                                  125

Gly Gly Gly Gly Ser Ala Leu Pro Val Leu Thr Gln Pro Pro Ser Ala  
 130 135 140  
 Ser Gly Ser Pro Gly Gln Ser Val Thr Ile Ser Cys Thr Gly Thr Ser  
 145 150 155 160  
 Ser Asp Val Gly Gly Tyr Lys Tyr Val Ser Trp Tyr Gln Gln His Pro  
 165 170 175  
 Gly Lys Ala Pro Lys Leu Met Ile Tyr Glu Val Ser Met Arg Pro Ser  
 180 185 190  
 Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser  
 195 200 205  
 Leu Thr Val Ser Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys  
 210 215 220  
 Ala Ser Tyr Ala Gly Ser Asn Asn Trp Val Phe Gly Gly Gly Thr Lys  
 225 230 235 240  
 Leu Thr Val Leu Gly  
 245

<210> 49

<211> 245

<212> PRT

<213> Artificial sequence

<220>

<223> T1014G04 scFv

<400> 49

Glu Val Gln Leu Val Gln Ser Gly Ala Asp Val Lys Arg Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ile Ser Gly Asp Ser Phe Thr Ala Tyr  
 20 25 30  
 Phe Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Trp Phe Asn Pro Asp Ser Gly Thr Ala Asp Ser Ala Gln Lys Phe  
 50 55 60  
 His Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ser Ser Thr Ala Phe  
 65 70 75 80  
 Leu Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Val Arg Gln His Arg Gly Asn Thr Phe Ala Pro Trp Gly Arg Gly Thr  
 100 105 110  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 115 120 125  
 Gly Gly Gly Gly Ser Ala Gln Pro Val Leu Thr Gln Pro Pro Ser Ala  
 130 135 140

Ser Gly Ser Pro Gly Gln Ser Val Thr Ile Ser Cys Thr Gly Thr Ser  
 145 150 155 160  
 Ser Asp Val Gly Ser Tyr Glu Tyr Val Ser Trp Tyr Gln Gln His Pro  
 165 170 175  
 Gly Lys Ala Pro Arg Leu Met Ile Ser Glu Val Asn Lys Arg Pro Ser  
 180 185 190  
 Gly Val Pro Asn Arg Phe Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser  
 195 200 205  
 Leu Thr Val Ser Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys  
 210 215 220  
 Ser Ser Tyr Ala Gly Ser Asn Asn Trp Val Phe Gly Gly Gly Thr Lys  
 225 230 235 240  
 Val Thr Val Leu Gly  
 245

<210> 50

<211> 250

<212> PRT

<213> Artificial sequence

<220>

<223> T1015A02 scFv

<220>

<221> SITE

<222> (250)

<223> Xaa equals either Gly or Ser

<400> 50

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln  
 1 5 10 15  
 Thr Leu Ser Leu Lys Cys Asn Val Ser Gly Gly Ser Ile Gly Thr Gly  
 20 25 30  
 Asp Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu  
 35 40 45  
 Trp Ile Gly Tyr Ile His Ser Ser Gly Ser Thr Tyr Tyr Lys Pro Ser  
 50 55 60  
 Leu Arg Ser Arg Leu Thr Val Ser Met Asp Thr Ser Arg Asn Gln Phe  
 65 70 75 80  
 Ser Leu Lys Leu Thr Ser Val Thr Ala Ala Asp Thr Ala Leu Tyr Tyr  
 85 90 95  
 Cys Val Arg Glu Trp Ala Asn Gly Asp His Trp Ser Ala Phe Asp Leu  
 100 105 110  
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser  
 115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Ala Val Leu Thr  
 130 135 140  
 Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln Arg Val Thr Ile Pro  
 145 150 155 160  
 Cys Ser Gly Ser Ser Ser Asn Ile Gly Gly Asn Thr Val Asn Trp Tyr  
 165 170 175  
 Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Gly Asn Asp  
 180 185 190  
 Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly  
 195 200 205  
 Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln Ser Glu Asp Glu Ala  
 210 215 220  
 Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu Ile Gly Tyr Val Phe  
 225 230 235 240  
 Gly Thr Gly Thr Gln Leu Thr Val Leu Xaa  
 245 250

<210> 51  
 <211> 245  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> T1015A07 scFv

<400> 51  
 Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ile Ser Gly Asp Ser Phe Thr Ala Tyr  
 20 25 30  
 Phe Ile His Trp Leu Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Met  
 35 40 45  
 Gly Trp Phe Asn Pro Ile Ser Gly Thr Ala Asp Ser Pro Gln Lys Phe  
 50 55 60  
 His Gly Arg Val Ala Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Thr Arg Leu Ala Ser Asp Asp Thr Ala Ile Tyr Tyr Cys  
 85 90 95  
 Ala Arg Gln His His Ser Asn Thr Phe Asp Pro Trp Gly Gln Gly Thr  
 100 105 110  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 115 120 125  
 Gly Gly Gly Gly Ser Ala Gln Ser Ala Leu Thr Gln Pro Ala Ser Met  
 130 135 140



Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser  
 145 150 155 160  
 Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr Gln Gln His Pro  
 165 170 175  
 Gly Lys Ala Pro Lys Leu Met Ile Tyr Ala Val Thr Asn Arg Pro Ser  
 180 185 190  
 Gly Val Ser Asn Arg Phe Ser Ala Ser Lys Ser Gly Asn Thr Ala Ser  
 195 200 205  
 Leu Thr Ile Ser Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys  
 210 215 220  
 Ser Ser Tyr Thr Ser Ser Asn Thr Trp Val Phe Gly Gly Gly Thr Lys  
 225 230 235 240  
 Val Thr Val Leu Gly  
 245

<210> 52  
 <211> 245  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> T1015E01 scFv

<220>  
 <221> SITE  
 <222> (4)  
 <223> Xaa equals Val or Leu

<220>  
 <221> SITE  
 <222> (5)  
 <223> Xaa equals Ala or Val

<220>  
 <221> SITE  
 <222> (7)  
 <223> Xaa equals Ala or Ser

<220>  
 <221> SITE  
 <222> (10)  
 <223> Xaa equals Asp or Glu

<220>  
 <221> SITE  
 <222> (12)  
 <223> Xaa equals Asn or Lys

<220>  
 <221> SITE  
 <222> (23)  
 <223> Xaa Met or Lys

&lt;400&gt; 52

Glu Val Gln Xaa Xaa Gln Xaa Gly Ala Xaa Val Xaa Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Xaa Ile Ser Gly Asp Ser Phe Thr Ala Tyr  
 20 25 30  
 Phe Ile His Trp Leu Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Met  
 35 40 45  
 Gly Trp Phe Asn Pro Ile Ser Gly Thr Ala Asp Ser Pro Gln Lys Phe  
 50 55 60  
 His Gly Arg Val Ala Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Thr Arg Leu Ala Ser Asp Asp Thr Ala Ile Tyr Tyr Cys  
 85 90 95  
 Ala Arg Gln His His Ser Asn Thr Phe Asp Pro Trp Gly Gln Gly Thr  
 100 105 110  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 115 120 125  
 Gly Gly Gly Gly Ser Ala Gln Ser Ala Leu Thr Gln Pro Ala Ser Met  
 130 135 140  
 Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser  
 145 150 155 160  
 Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr Gln Gln His Pro  
 165 170 175  
 Gly Lys Ala Pro Lys Leu Met Ile Tyr Ala Val Thr Asn Arg Pro Ser  
 180 185 190  
 Gly Val Ser Asn Arg Phe Ser Ala Ser Lys Ser Gly Asn Thr Ala Ser  
 195 200 205  
 Leu Thr Ile Ser Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys  
 210 215 220  
 Ser Ser Tyr Thr Ser Ser Asn Thr Trp Val Phe Gly Gly Gly Thr Lys  
 225 230 235 240  
 Val Thr Val Leu Gly  
 245

&lt;210&gt; 53

&lt;211&gt; 249

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; T1006F07 scFv

&lt;400&gt; 53

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
                   20                                  25                                  30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
                   35                                  40                                  45  
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
                   50                                  55                                  60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
                   65                                  70                                  75                                  80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
                                   85                                  90                                  95  
 Ala Arg Glu Pro Ser Phe Gln Gln Trp Gly His Tyr Ser Tyr Gly Met  
                                   100                                  105                                  110  
 Asp Val Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Gly Gly Gly  
                   115                                  120                                  125  
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Ser Val  
                   130                                  135                                  140  
 Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln Ala Ala Arg  
                   145                                  150                                  155                                  160  
 Ile Thr Cys Ser Gly Asp Lys Leu Gly Asp Lys Tyr Ala Ser Trp Tyr  
                                   165                                  170                                  175  
 Gln Gln Arg Pro Gly Gln Ser Pro Val Leu Val Ile Tyr Gln Asp Asn  
                                   180                                  185                                  190  
 Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly  
                   195                                  200                                  205  
 Asn Thr Ala Thr Leu Lys Ile Ser Gly Thr Gln Ala Met Asp Glu Ala  
                   210                                  215                                  220  
 Asp Tyr Tyr Cys Leu Ala Trp Asp Ser Ser Ala Asp Trp Val Phe Gly  
                   225                                  230                                  235                                  240  
 Gly Gly Thr Lys Val Thr Val Leu Gly  
                                   245

&lt;210&gt; 54

&lt;211&gt; 735

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014A04 scFv

&lt;400&gt; 54

gaggtgcagc tgggtgcagtc tggggctgac gtgaagaggc ctggggcctc agtgaaggtc 60  
 tcttgcaaga tttctggaga cagcttcaac gcctacttta ttcactgggt gcgtcaggcc 120  
 cctggacagg ggcttgagtg gatgggatgg ttcaaccctg acagtggtag cgcagactct 180  
 gcacagaagt ttcacggcag ggtcaccatg accagggaca cgtccagcag tactgccttc 240

```

ttggagctga gcagactgag atctgacgac acagccgtgt attactgtgt gagacaacat 300
cggggtaaca cgttcgcccc ctggggccgg gggacaatgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcgagggttg ctctggcggg ggcggaagtg cacagtctgt gctgactcag 420
ccaccctccg cgtccgggtc tcctggacag tcagtcacca tctcctgcac tggaaccacc 480
agtgacgttg gtggttataa ctatgtctcc tggtagcaac agcaccaggg caaagccccc 540
aaactcatga tttatggggg caatcagcgg ccctcagggg tccctgatcg cttctctggc 600
tccaagtctg gcaacacggc ctccctgacc gtctctgggc tccaggctga ggatgaggct 660
gattattact gcagttcata tgcaggcagc aacaattggg tgttcggcgg agggaccaag 720
ctgaccgtcc taggt 735

```

&lt;210&gt; 55

&lt;211&gt; 735

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014G03 scFv

&lt;400&gt; 55

```

gaggtccagc tggtagagtc tggagctgaa gtgaagatgc ctggggcctc agtcaagctc 60
tcctgcaggg tttctggaga caccctcacc gcctacttca ttcactgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggatgg ttcaacccta tcagtggcac cgcaggctct 180
gctgagaagt ttccggcgag ggtcgccatg accagggaca cgtccatcag cactgcctac 240
atggaattga acaggctgac atttgacgac acggccgtct attattgtgc gagacaacat 300
cgggggaata cgtttgaccc ctggggccag ggcaccctgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcgagggttg ctctggcggg ggcggaagtg cacagtctgc cctgactcag 420
cctgcctccg tgtctgggtc tcctggacag tcgatcacca tctcctgcac tggaaccagg 480
agtgcatttg gtgcttataa gtatgtctcc tggtagcaac aacaccaggg caaagccccc 540
aaacttgtga tttatgaggt cagtaatcgg ccctcagggg tttccagtcg cttctctggc 600
tccaagtctg gccagacggc ctccctgacc atctctgggc tccaggctga cgacgaggct 660
gattattact gcaactcata tcaaggttac aacacgtggg tgttcggcgg agggaccaag 720
gtcaccgtcc taggt 735

```

&lt;210&gt; 56

&lt;211&gt; 732

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014A02 scFv

&lt;400&gt; 56

```

caggtgcagc tgcaggagtc cggcccagga ctggtgaagc cctcggagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagt gattactact ggagttgggt ccggcagtc 120
cccgggaagg gactggagtg gattgggtct atcgattatg ccggcagcac caattacaac 180
ccgtccctca agagccgagt caccatgaca atagacaagt ccaagaagca attccccctg 240
aagatagatt ctgtgaccgc cgcagatacg gccatgtatt actgtgcgag acaacttggg 300
cggatttctg actactgggg ccagggcacc ctggtcaccg tctcgagtgg aggcggcggg 360
tcaggcggag gtggctctgg cgggtggcga agtgcacttt cctatgtgct gactcagcca 420
ccctcagcgt ctgggacccc cgggcagagg gtcaccatct cttgtgctgg aagcagctcc 480
aacatcggag gaaatactgt aaactggtac cagcaactcc cagcaacggc ccccaaactc 540
ctcatctata gtaataatca gcggccctca ggggtccctg accgattctc tggctccaag 600
tctggcacgt cagcctccct ggccatcagt gggctccagt ctgaggatga ggctgattat 660
tactgtgcaa catgggatga cagtcggggg ggttgggtgt tcggcggagg gaccaagctg 720
accgtcctag gt 732

```

&lt;210&gt; 57

&lt;211&gt; 735

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014A12 scFv

&lt;400&gt; 57

```

gaggtccagc tgggtgcagtc tgggggctgac gtgaagaggc ctgggggcctc agtgaaggtc 60
tcctgcaaga tttctggaga cagcttcacc gcctacttta ttcactgggt gcgtcaggcc 120
cctggacagg ggcttgagtg gatgggatgg ttcaaccctg acagtggtag cgcagactct 180
gcacagaagt ttcacggcag ggtcaccatg accagggaca cgtccagcag tactgccttc 240
ttggagctga gcagactgag atctgacgac accgcccgtat attactgtgt gagacaacat 300
cggggtaaca cgttcgcccc ctggggccgg gggacaatgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcgagagtggt ctctggcggt ggcggaagtg cacagtctgc cctgactcag 420
cctgcctccg tgtctgggtc tcctggacag tcgatcacca tctcctgcac tggatccagc 480
agtgcagttg gtgggtataa gtatgtctcc tggtagcaaac aacacccagg caaagcccc 540
aaactcatta ttcattgatgt cagtaggcgg ccctcagagg tttctagtcg cttctctggc 600
tccaagtctg gcaacacggc ctccctgacc atctctgggc tccaggctga ggacgaggct 660
gagtactact gcagctcata ttcaagcacc aactcttggg tgttcggcgg agggaccaag 720
gtcaccgtcc taggt                                     735

```

&lt;210&gt; 58

&lt;211&gt; 735

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014B01 scFv

&lt;400&gt; 58

```

caggtccagc tgggtgcagtc tgggggctgag gtgaagaagc ctgggggcctc agtgaaggtc 60
tcctgcaaga tttctggaga caccttcgcc gcctacttta ttcactgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggatgg ttcaacccta acagtggtag cgcagactct 180
tcacagaagt ttcacggcag ggtcaccatg accagggaca cgtccatcag cactgcctac 240
atggagttga gcaggctgag atctgacgac acggccgtgt attattgtgc gagacaacat 300
cgggtctaata cgttcgaccc ctggggccaa gggacaatgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcgagagtggt ctctggcggt ggcggaagtg cacagtctgt cgtgacgcag 420
ccgccctcag tgtctgggtc tcctggacag tcagtcacca tctcctgcac tgggaaccagc 480
agtgcacattg gtgcttataa ttatgtctcc tggttccagc agcaccagg taaagcccc 540
aaactcataa tttctgaggt cagtaagcgg ccctcagggg tccctgatcg cctctctggc 600
tccaagtctg gcaacacggc ctccctgacc gtctccgggc tccaggctga ggatgaggct 660
gattattact gcggctcata tgcaggcagc aatatttggg tgttcggcgg agggaccaag 720
gtcaccgtcc taggt                                     735

```

&lt;210&gt; 59

&lt;211&gt; 735

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding T1014B11 scFv

&lt;400&gt; 59

```

gaggtccagc tgggtgcagtc tgggggctgag gtgaagaagc ctgggggcctc agtaaaggctc 60
tcctgcaaga tttctggaga cagcttcacc gcctatttta ttcactgggt gcgacaggcc 120
cctggagaag ggcttgagtg gatgggatgg ttcaatccta tcagcggtag cgcgggtctc 180
ccacagaagt ttcacggcag ggtcgccatg acccgtgaca cgtccatcag tactgcctac 240
atggagttga ccaggctggc atctgacgac acggccattt attattgtgc gagacaacat 300
cactctaata cgttcgaccc ctggggccaa ggaaccctgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcgagagtggt ctctggcggt ggcggaagtg cacaatctgc cctgactcag 420
cctgcctccg tgtctgggtc tcctggacag tcgatcacca tctcctgcac tggaaaccaac 480
agtgcagttg gtgggtataa ctatgtctcc tggtagcaaac aacacccagg caaagcccc 540

```

```

aaactcatga tttatgaggt caataatcgg ccctcagggg tttctaatacg cttctctggc 600
tccaagtctg gcaacacggc ctccctgacc atctctgggc tccaggctga cgacgaggct 660
gattattact gcagctcata tacaaccagc aacacttggg tggttcggcgaggaggaccaag 720
ctgaccgtcc taggt 735

```

<210> 60

<211> 735

<212> DNA

<213> Artificial sequence

<220>

<223> DNA encoding T1014F08 scFv

<400> 60

```

gaggtccagc tgggtgcagtc tggggctgaa gtgaagaagc ctggggcctc agtcaagctc 60
tcctgcaggg tttctggaga caccttcacc gcctacttca ttcactgggt gcgacaggcc 120
cctggacaag ggcctgagtg gatgggatgg ttcaacccta tcagtggcac cgcaggctct 180
gctgcgaggt ttgcggcag ggtcgccatg accagggaca cgtccatcag cactgcctac 240
atggaattga acaggctgac atttgacgac acggccgtct attattgtgc gagacaacat 300
cgggggaata cctttgaccc ctggggcaaa ggcaccctgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcggaggtgg ctctggcggg ggcggaagtg cactgcctgt gctgactcag 420
ccaccctccg cgtccgggtc tcctggacag tcagtcacca tctcctgcac tggaaccagc 480
agtgcggttg gtggttataa gtatgtctcc tggtaccaac agcaccagc caaagccccc 540
aaactcatga tttatgaggt cagtatgcgg ccgtcagggg tcccggatcg cttctctggc 600
tccaagtctg gcaacacggc ctccctgacc gtctctgggc tccaggctga ggatgaggct 660
gattattact gcgcctcata tgcaggcagc aacaattggg tggttcggcgaggaggaccaag 720
ctgaccgtcc taggt 735

```

<210> 61

<211> 735

<212> DNA

<213> Artificial sequence

<220>

<223> DNA encoding T1014G04 scFv

<400> 61

```

gaagtgcagc tgggtgcagtc tggggctgac gtgaagaggc ctggggcctc agtgaaggctc 60
tcctgcaaga tttctggaga cagcttcacc gcctacttta ttcactgggt gcgtcaggcc 120
cctggacagg ggcctgagtg gatgggatgg ttcaaccctg acagtgggtac cgcagactct 180
gcacagaagt ttcacggcag ggtcaccatg accagggaca cgtccagcag tactgccttc 240
ttggagctga gcagactgag atctgacgac accgcgtat attactgtgt gagacaacat 300
cggggtaaca cgttcgcccc ctggggcagg ggaaccctgg tcaccgtctc gagtggaggc 360
ggcgggttcag gcggaggtgg ctctggcggg ggcggaagtg cacagcctgt gctgactcag 420
ccccctccg cgtccgggtc gcctggacag tcagtcacca tctcctgcac tggaaccagc 480
agtgcggttg gtagttatga gtatgtctcc tggtaccaac aacaccagc caaagccccc 540
agactcatga tttctgaggt caataagcgg ccctcagggg tccctaatacg cttctctggc 600
tccaagtctg gcaacacggc ctccctgacc gtctctgggc tccaggctga cgatgaggct 660
gattactact gcagctcata tgcaggcagc aacaattggg tggttcggcgaggaggaccaag 720
gtcaccgtcc taggt 735

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(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
5 December 2002 (05.12.2002)

PCT

(10) International Publication Number  
**WO 2002/097033 A3**

(51) International Patent Classification<sup>7</sup>: **A61K 39/42**,  
39/395, C07K 16/00, C12N 5/12

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(21) International Application Number:  
PCT/US2002/014268

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(22) International Filing Date: 7 May 2002 (07.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/293,473	25 May 2001 (25.05.2001)	US
60/294,981	4 June 2001 (04.06.2001)	US
60/309,176	2 August 2001 (02.08.2001)	US
60/323,807	21 September 2001 (21.09.2001)	US
60/327,364	9 October 2001 (09.10.2001)	US
60/331,044	7 November 2001 (07.11.2001)	US
60/331,310	14 November 2001 (14.11.2001)	US
60/341,237	20 December 2001 (20.12.2001)	US
60/369,860	5 April 2002 (05.04.2002)	US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,  
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,  
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent  
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
NE, SN, TD, TG).

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Published:

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

(88) Date of publication of the international search report:  
20 October 2005

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: ANTIBODIES THAT IMMUNOSPECIFICALLY BIND TO TRAIL RECEPTORS

(57) Abstract: The present invention relates to antibodies and related molecules that immunospecifically bind to TRAIL receptor, TR4. Such antibodies have uses, for example, in the prevention and treatment of cancers and other proliferative disorders. The invention also relates to nucleic acid molecules encoding anti-TR4 antibodies, vectors, and host cells containing these nucleic acids, and methods for producing the same. The present invention relates to methods and compositions for preventing, detecting, diagnosing, treating, or ameliorating a disease or disorder, especially cancer and other hyperproliferative disorders, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to TRAIL receptor TR4.



WO 2002/097033 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/14268

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/42, 39/395; C07K 16/00; C12N 5/12  
US CL : 424/139.1, 155.1; 435/334, 344; 530/388.8, 391.3

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 424/139.1, 155.1; 435/334, 344; 530/388.8, 391.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,T	WANG S. et al. Requirement of p53 targets in chemosensitization of colonic carcinoma to death ligand therapy. Proc. Natl. Acad. Sci. USA. 09 December 2003; Vol. 100, No. 25, pages 15095-15100; see entire document.	1-6, 9-12, 15-54, 65, 66, 72-74, and 76
Y,E	US 2003/0190685 A1 (SALCEDO et al.) 09 October 2003 (09.10.203); see entire document.	1-6, 9-12, 15-54, 65, 66, 72-74, and 76
A	CHUNTHARAPAI, A. et al. Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4. J. Immunol. 15 April 2001; Vol 166, No. 8, pages 4891-4898; see entire document.	1-6, 9-12, 15-54, 65, 66, 72-74, and 76
A,T	VAN GEELEN, CM. et al. Differential modulation of the TRAIL receptors and the CD95 receptor in colon carcinoma cell lines. Br. J. Cancer. 21 July 2003; Vol. 89, No. 2, pages 363-373; see entire document.	1-6, 9-12, 15-54, 65, 66, 72-74, and 76
A,T	NAKA, T. et al. Effects of tumor necrosis factor-related apoptosis-inducing ligand alone and in combination with chemotherapeutic agents on patients' colon tumors growth in SCID mice. Cancer Res. 15 October 2002; Vol. 62, No. 20, pages 580-5806; see entire document.	1-6, 9-12, 15-54, 65, 66, 72-74, and 76

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 January 2004 (28.01.2004)

Date of mailing of the international search report

19 AUG 2005

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Stephen L. Rawlings, Ph.D.

Telephone No. 571-272-1600

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/14268

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: Please See Continuation Sheet

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

PCT/US02/14268

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-6, 9-12, 15-66, 72-74, and 76, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 42, a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42, a kit comprising said antibody or fragment thereof, and the antibody expressed by the cell line of ATCC Deposit No. PTA-3571.

Group II, claim(s) 1-5, 7, 9-11, 13, 15-66, 72-74, and 75, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 43, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43, and the antibody expressed by the cell line of ATCC Deposit No. PTA-3570.

Group III, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 44, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

Group IV, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 45, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

Group V, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 46, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

Group VI, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 47, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

Group VII, claim(s) 1-5, 8-11, 14, 15-66, 72-74, and 77, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 48, a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48, and the antibody expressed by the cell line of ATCC Deposit No. PTA-3675.

Group VIII, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 49, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group IX, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 50, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group X, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 51, and a

# INTERNATIONAL SEARCH REPORT

PCT/US02/14268

kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

Group XI, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 52, and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group XII, claim(s) 1-5, 9-11, 15-66, and 72-74, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 53 and a kit comprising an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Group XIII, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

Group XIV, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

Group XV, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

Group XVI, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

Group XVII, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

Group XVIII, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

Group XIX, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group XX, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group XXI, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

Group XXII, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group XXIII, claim(s) 52-66, insofar as the claims are drawn to a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Group XXIV, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating graft vs. host disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.



Group LTV, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.



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Group LV, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group LVI, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group LVII, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

Group LVIII, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group LIX, claim(s) 67 and 68, insofar as the claims are drawn to a method for treating, preventing or ameliorating a neurodegenerative disease comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Group LX, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

Group LXI, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

Group LXII, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

Group LXIII, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

Group LXIV, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

Group LXV, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

Group LXVI, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

Group LXVII, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group LXVIII, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group LXIX, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

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Group LXX, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group LXXI, claim(s) 69, insofar as the claims are drawn to a method for inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Group LXXII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

Group LXXIII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

Group LXXIV, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

Group LXXV, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

Group LXXVI, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

Group LXXVII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

Group LXXVIII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

Group LXXIX, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group LXXX, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group LXXXI, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

Group LXXXII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group LXXXIII, claim(s) 70, insofar as the claims are drawn to a method for detecting the expression of a TR4 polypeptide comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Group LXXXIV, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

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Group LXXXV, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

Group LXXXVI, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

Group LXXXVII, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

Group LXXXVIII, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

Group LXXXIX, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

Group XC, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

Group XCI, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

Group XCII, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

Group XCIII, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

Group XCIV, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

Group XCV, claim(s) 71, insofar as the claims are drawn to a method for detecting, prognosing, or monitoring cancers or other hyperproliferative disorders comprising assaying the expression of said polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Claim 52 is generic to a plurality of species of invention wherein said cancer is selected from the group consisting of (a) colon cancer, (b) breast cancer, (c) uterine cancer, (d) pancreatic cancer, (e) lung cancer, (f) gastrointestinal cancer, (g) Kaposi's sarcoma, and (h) central nervous system cancer.

Claim 61 is generic to a plurality of species of invention wherein said central nervous system cancer is selected from the group consisting of (i) medulloblastoma, (j) neuroblastoma, and (k) glioblastoma.

Claim 65 is generic to a plurality of species of invention wherein said chemotherapeutic agent is selected from the group consisting of (l) irinotecan, (b) paclitaxel, and (c) gemcitabine.

The inventions listed as Groups I-XCV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

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The special technical feature of group I is the scFv antibody of SEQ ID NO: 42.

The special technical feature of group II is the scFv antibody of SEQ ID NO: 43.

The special technical feature of group III is the scFv antibody of SEQ ID NO: 44.

The special technical feature of group IV is the scFv antibody of SEQ ID NO: 45.

The special technical feature of group V is the scFv antibody of SEQ ID NO: 46.

The special technical feature of group VI is the scFv antibody of SEQ ID NO: 47.

The special technical feature of group VII is the scFv antibody of SEQ ID NO: 48.

The special technical feature of group VIII is the scFv antibody of SEQ ID NO: 49.

The special technical feature of group IX is the scFv antibody of SEQ ID NO: 50.

The special technical feature of group X is the scFv antibody of SEQ ID NO: 51.

The special technical feature of group XI is the scFv antibody of SEQ ID NO: 52.

The special technical feature of group XII is the scFv antibody of SEQ ID NO: 53.

The special technical feature of group XIII is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group XIV is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group XV is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

The special technical feature of group XVI is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group XVII is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group XVIII is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

The special technical feature of group XIX is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group XX is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group XXI is treating, preventing or ameliorating a cancer by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

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The special technical feature of group XXXVI is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

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The special technical feature of group XXXVII is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group XXXVIII is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group XXXIX is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

The special technical feature of group XL is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group XLI is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group XLII is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

The special technical feature of group XLIII is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group XLIV is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group XLV is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

The special technical feature of group XLVI is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

The special technical feature of group XLVII is treating, preventing or ameliorating AIDS by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

The special technical feature of group XLVIII is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

The special technical feature of group XLIX is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group L is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group LI is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

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The special technical feature of group LII is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group LIII is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group LIV is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

The special technical feature of group LV is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group LVI is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group LVII is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

The special technical feature of group LVIII is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

The special technical feature of group LIX is treating, preventing or ameliorating a neurodegenerative disease by a process comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

The special technical feature of group LX is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

The special technical feature of group LXI is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group LXII is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group LXIII is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

The special technical feature of group LXIV is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group LXV is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group LXVI is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

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The special technical feature of group LXVII is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group LXVIII is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group LXIX is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

The special technical feature of group LXX is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

The special technical feature of group LXXI is inhibiting the growth of or killing TR4 expressing cells comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

The special technical feature of group LXXII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.

The special technical feature of group LXXIII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group LXXIV is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group LXXV is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

The special technical feature of group LXXVI is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group LXXVII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group LXXVIII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

The special technical feature of group LXXIX is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group LXXX is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group LXXXI is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

The special technical feature of group LXXXII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

The special technical feature of group LXXXIII is detecting the expression of TR4 polypeptide using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

The special technical feature of group LXXXIV is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42.



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The special technical feature of group LXXXV is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 43.

The special technical feature of group LXXXVI is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 44.

The special technical feature of group LXXXVII is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 45.

The special technical feature of group LXXXVIII is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 46.

The special technical feature of group LXXXIX is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 47.

The special technical feature of group XC is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 48.

The special technical feature of group XCI is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 49.

The special technical feature of group XCII is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 50.

The special technical feature of group XCIII is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 51.

The special technical feature of group XCIV is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 52.

The special technical feature of group XCV is detecting, diagnosing, prognosing, or monitoring cancers and other hyperproliferative disorders using an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 53.

Inventions of groups I-XCV do not share the same or corresponding special technical feature so as to form a single general inventive concept under PCT Rules 13.1 and 13.1; and PCT Rules 13.1 and 13.2 do not provide for a single general inventive concept comprising more than the first mentioned product, the first mentioned method for making said product, and the first mentioned method for using said product.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The special technical feature of species A is treating, preventing or ameliorating colon cancer.

The special technical feature of species B is treating, preventing or ameliorating breast cancer,

The special technical feature of species C is treating, preventing or ameliorating uterine cancer,

The special technical feature of species D is treating, preventing or ameliorating pancreatic cancer,

The special technical feature of species E is treating, preventing or ameliorating lung cancer,

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The special technical feature of species F is treating, preventing or ameliorating gastrointestinal cancer.

The special technical feature of species G is treating, preventing or ameliorating Kaposi's sarcoma.

The special technical feature of species H is treating, preventing or ameliorating a cancer of the central nervous system, namely a medulloblastoma.

The special technical feature of species I is treating, preventing or ameliorating a cancer of the central nervous system, namely a neuroblastoma,

The special technical feature of species J is treating, preventing or ameliorating a cancer of the central nervous system, namely a glioblastoma.

Accordingly, species A, B, C, D, E, F, G, H, I, and J do not share the same or corresponding special technical feature so as to form a single general inventive concept under PCT Rules 13.1 and 13.1.

The special technical feature of species K is administering to an animal irinotecan.

The special technical feature of species L is administering to an animal paclitaxel.

The special technical feature of species M is administering to an animal gemcitabine.

Accordingly, species K, L, and M do not share the same or corresponding special technical feature so as to form a single general inventive concept under PCT Rules 13.1 and 13.1.

## **Continuation of Box II Item 4:**

1-6, 9-12, 15-66, 72-74, and 76, insofar as the claims are drawn to an antibody, or fragment thereof, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of at least a CDR of the scFv antibody of SEQ ID NO: 42, a method for treating, preventing or ameliorating a cancer comprising administering to an animal an antibody, or fragment thereof, comprising an amino acid sequence that is at least 90% identical to at least a VH or VL domain of the scFv antibody of SEQ ID NO: 42, and optionally comprising administering to said animal a chemotherapeutic agent, wherein said cancer is colon cancer and said chemotherapeutic agent is irinotecan, a kit comprising said antibody or fragment thereof, and the antibody expressed by the cell line of ATCC Deposit No. PTA-3571.

## **Continuation of B. FIELDS SEARCHED Item 3:**

Databases MEDLINE, WEST, ISSUED PATENTS, PUBLISHED APPLICATIONS, GENEMBL, GENESEQ, SPTREMBL, SWISS PROTEIN, PIR: SEQ ID NO: 42; antibody, TRAIL Receptor-4, DR4, TRAIL-R4, irinotecan, colon cancer, immunotherapy, adjuvant therapy.